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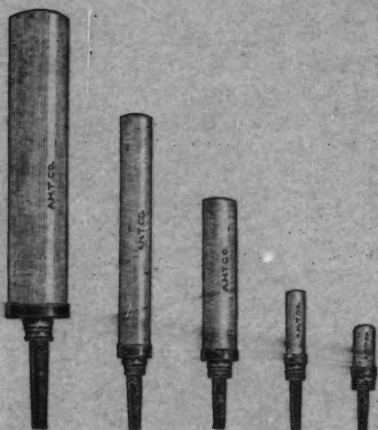
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STUDIES ON THE GROWTH OF MAN

III. THE GROWTH OF BRITISH INFANTS DURING THE FIRST YEAR SUCCEEDING BIRTH

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Received for publication, August 30, 1916

1. OBJECT OF THE INVESTIGATION AND SOURCES OF DATA

While we now possess a fairly accurate and extensive knowledge of the normal curve of growth of certain animals, particularly the white rat and the white mouse, it is a regrettable fact that we are not yet in possession of equally reliable and extensive data concerning the growth of human beings. While we know that growth in human beings as in mice takes place in three "cycles" or periods of alternately rapid and slow growth (1), we do not yet possess, for any one race of mankind, a detailed knowledge of the extent and characteristics of each of these cycles. We possess in several instances very extensive knowledge of portions of the total curve, and if it were permissible to add together data obtained from different countries we would possess a sufficiency of information to enable us to construct in detail the entire growth-curve of man. But the most superficial comparison of data from different countries, as for example those of the British Anthropometric Committee (2) with those obtained by Quetelet in Belgium (3) at once reveals the fact that the growth-curves of different races of man or even of similar races inhabiting different localities (4) are by no means identical and that parts of the growth-curve constructed from measurements made in France for example cannot therefore be legitimately regarded as continuations of other parts of the human growth-curve constructed from measurements made in England.

As regards the inhabitants of Great Britain we possess, in the various reports of the Anthropometric Committee (5), the Royal Commission on Physical Training (Scotland) (6), the Scotch Education Department (7), the medical officer of health of the city of Glasgow (8) and the chief medical officer of the Board of Education (9), a very complete picture of their growth from the beginning of school age onwards. Similar data regarding growth from birth to school age appear, however, to be lacking.

The medical advisors of the various child welfare leagues in Great Britain appear usually to regard the so-called "Newman Standard" as representing the growth of British infants during the first two years succeeding birth, and charts representing this standard are not unusually displayed in the offices of these organizations or even plotted for purposes of comparison upon the growth-charts of the infants under their care. I was unable, however, by inquiry amongst the officials and medical advisors of these institutions in London to ascertain the source of this standard, and therefore addressed my inquiry directly to Sir George Newman, who very kindly replied, referring me to his work on Infant Mortality (10). In that work, however, although charts of the "Standard" are supplied, the source of the measurements from which the charts are constructed is nowhere clearly indicated, unless indeed it be upon page 303 where the author states: "Dufour's Standard has been used in the Finsbury Dépôt as in the French Dépôts and the charts appearing in the present volume are drawn to that scale."

The standard of infant growth employed in England would therefore appear to have originated in France. Having regard to the very marked influence of locality upon human growth, as displayed in the discrepancy between the weight of British infants born in Australia and those of infants born in the British Isles (11) and adding thereto the influence, to which reference has been made above, of race upon growth, it is very evident that a French standard cannot be safely relied upon as representing the normal growth of British infants, and marked discrepancies between the average weights of British infants and those indicated by the "Newman Standard" may therefore be anticipated. Moreover the Newman Standard makes no distinction between the sexes, whereas as the above cited reports and many observations upon animals show, the growth-curves of the two sexes, even during the first growth-cycle, differ from one another very considerably.

That marked discrepancies exist between the actual normal weights of British infants and the Newman Standard weights has been pointed out by Pooler (12).

It appeared, therefore, in order to construct this portion of the growth-curve of the inhabitants of Great Britain, necessary to obtain fresh estimates of the average weights of children at monthly intervals during the first two years succeeding birth. I accordingly, in the spring of 1914, addressed myself to the medical advisors and other officials of the various child-welfare institutions in London, Leeds and Bristol and in several instances obtained permission, which was very greatly appreciated, to obtain access to and to copy the valuable data collected by these institutions during the execution of their activities. For assistance in obtaining these data I am especially indebted to the following persons to whom I wish to take this opportunity of expressing my obligations:

Drs. Eric Pritchard, David Forsyth and R. C. Jewsbury in London, Drs. John Robertson and H. W. Pooler in Birmingham, Dr. Leslie Mackenzie in Edinburgh, Dr. James Smart in Aberdeen, Mrs. Ogilvie, honorary secretary of the Chelsea Health Society; Miss Margaret Horn, honorary organizer of the city of Westminster Health Society. Mrs. Kitson Clark, president of the Leeds Babies Welcome, Miss E. Mozley, honorary secretary, Leeds Babies Welcome; Miss M. Curtis, superintendent Leeds Babies Welcome and F. M. Townsend, chairman of the Bristol Infant Welfare Association.

The data reported in this article were obtained from the records of the Pimlico Road and Golden Square branches of the Westminster Health Society and the Chelsea Health Society in London and from the Leeds Babies Welcome. The data obtained from other cities proved to be too fragmentary to utilize in statistical studies of this type.

In the case of the London institutions mentioned I was given the opportunity of personally inspecting the methods employed in weighing and examining the children and in keeping the records. In certain institutions which I visited no records of sex were kept and their data could not, therefore, be utilized. Other institutions only weighed the infants very approximately, and these data likewise were rejected. In the institutions mentioned, however, the sex of the infant is indicated upon the record and the weights are recorded to the nearest ounce avoirdupois. The infants were voluntarily brought by their mothers to the offices where a medical advisor and nurses or other trained officials were in attendance, the weight and condition of the

child being noted at each visit upon its record. The weights thus recorded were tabulated and averaged in the manner employed in the investigations previously reported (13). The weights of all infants reported as suffering from measles, whooping cough, chicken-pox, scarlet fever, diphtheria, pneumonia, bronchitis (unless specifically noted as "slight"), rachitis (unless specifically noted as "slight") and marasmus were excluded upon and succeeding the date upon which the condition was first noted. Data concerning twins and malformed children were likewise excluded and only children stated to be of British parentage on both sides and wholly or partially breast-fed (14) were accepted as sources of data. I was unable to personally visit the Leeds Babies Welcome, but the growth charts of a large number of infants were sent to me through the courtesy of the officials. The data as to disease included in these charts were not so full as in the London Records to which I had access and therefore although the charts furnished to me were specified as records of "normal children" it is possible that the selection was somewhat less rigorous in the case of the Leeds infants than in the case of the London infants. This may in part account for the slight discrepancy between the averages from the two sources during the first year of post-natal growth and for the very marked divergence of the two groups of children during the second year, to which attention will subsequently be drawn.

The data reported are exclusively ante-bellum, and were for the most part derived from records taken in the years 1911-1914. The delay in publication of the results arose from pressure of other work and from the large number of arithmetical computations involved.

2. RESULTS

The following tables 1 and 2 were the results obtained for infants during the first year of post-natal growth. The averages headed "England" are obtained by combining the data from London and Leeds, the variabilities at each age being calculated in the manner usually employed in statistical investigations (15).

The values of the Newman Standard are derived from the chart facing page 306 in Newman's Infant Mortality. On comparing the Standard with the results of these weighings it will be obvious that, as already noted by Pooler, the Newman Standard is considerably too low even for female infants.

TABLE 1

Males

AGE IN MONTHS	LONDON		LEEDS		ENGLAND			NEWMAN STANDARD
	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Variability	
							<i>per cent</i>	
1	48	149.0	38	144.4	86	147.0	17.0	123
2	69	172.9	48	163.4	117	169.0	16.4	142
3	69	197.1	65	190.3	134	193.8	15.8	161
4	48	227.3	66	212.3	114	218.6	15.9	180
5	52	244.8	72	225.8	124	233.7	14.7	204
6	38	266.0	67	243.7	105	251.8	15.1	228
7	28	283.4	62	262.0	90	268.6	14.9	244
8	34	289.4	60	268.7	94	276.2	13.1	255
9	24	286.7	61	281.2	85	282.8	13.8	268
10	21	320.2	46	290.4	67	299.7	13.4	282
11	24	313.8	37	296.6	61	303.4	15.3	295
12	19	342.6	41	301.2	60	314.3	12.6	308

TABLE 2

Females

AGE IN MONTHS	LONDON		LEEDS		ENGLAND			NEWMAN STANDARD
	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Variability	
							<i>per cent</i>	
1	44	143.7	36	142.4	80	143.1	14.4	123
2	67	159.1	47	160.0	114	159.5	15.0	142
3	66	179.1	57	181.0	123	180.0	15.6	161
4	56	201.9	48	202.2	104	202.1	15.0	180
5	41	221.2	49	214.5	90	217.6	14.5	204
6	34	240.3	49	232.0	83	235.4	13.4	228
7	30	258.7	46	248.6	76	252.6	14.6	244
8	33	268.5	46	251.2	79	258.4	14.0	255
9	27	268.1	53	264.0	80	265.4	14.0	268
10	22	274.2	35	272.7	57	273.3	11.6	282
11	22	301.5	28	277.4	50	288.0	12.5	295
12	25	304.7	31	273.6	56	287.5	11.9	308

The averages obtained from Leeds and from London are fairly concordant, the Leeds infants being as a rule slightly lighter than the London infants. The discrepancy, however, increases very markedly during the eleventh and twelfth months, and during the second year of post-natal growth, although an insufficient number of weighings to yield trustworthy averages was obtained, yet the Leeds infants were almost in every instance very markedly inferior in weight to the London infants. It is unquestionably not without significance that the deviation between the two groups of infants begins to be pronounced at the eleventh month after birth at which time according to

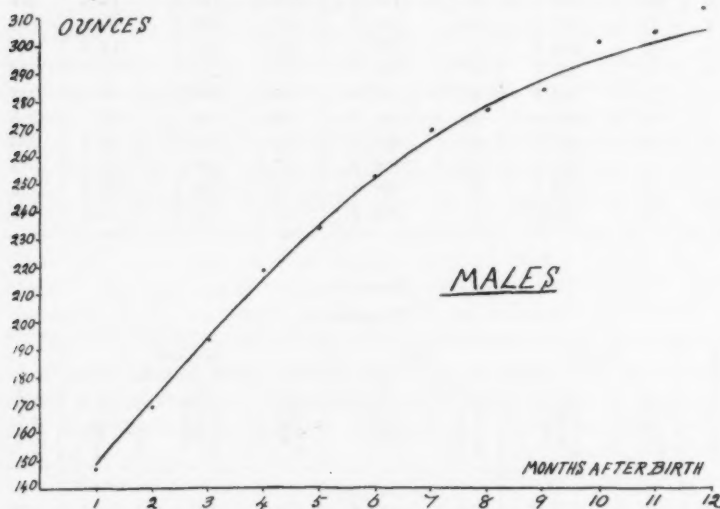


FIGURE 1.

Macgregor (16) there is a tendency for a certain percentage of infants to be of markedly subnormal weight, a tendency which I have elsewhere attributed (17) to a failure of the first and second growth-cycles to "link up" correctly.

The variability of male infants is greater than that of females and in both sexes the variability is greatest in the early months and falls off towards the end of the first year of post-natal growth.¹ The vari-

¹ The variabilities at eleven and twelve months of age would be considerably below the values recorded in tables 1 and 2 if it were not for the above-mentioned divergence between the London and Leeds groups.

ability is therefore greatest when the velocity of growth is greatest, a fact in harmony with data obtained in studying the growth of the white mouse (18).

It appears probable that high variability of weight, dimensions or other characteristics constitutes on the whole a condition unfavorable to the average welfare of any given group of organisms, since a high degree of variability implies more frequent possibility of overstepping physiological limits. It appears not unlikely therefore that the higher variability of male infants at birth (19) and subsequently to birth is correlated with the higher mortality among male infants at these ages (20).

3. THE FORMULATION OF THE CURVE OF GROWTH FOR BRITISH INFANTS DURING THE FIRST TWELVE MONTHS SUCCEEDING BIRTH

I have shown in previous communications (21) that the relationship between weight and age for any single growth-cycle is the same as that which subsists between the extent of transformation and the time in an autocatalysed chemical reaction, that is to say, a reaction one of the products of which accelerates it. The formula expressing this relationship is:

$$\log \frac{x}{A-x} = K(t-t_1)$$

where A is a constant (= the maximum weight attained by the particular growth-cycle under consideration), t_1 is a constant (= time at which the growth-cycle is half-completed) K is a constant (= kA where k is the specific velocity-constant and A has the value assigned above) and x and t are the weight and time respectively.

Applying this formula to the above data, utilizing for this purpose the methods and tables described in my "Tables for the Computation of Curves of Autocatalysis, with Especial Reference to Curves of Growth" (22) we find that for British males, during the first nine months of extra-uterine growth² the equation becomes:

$$\log_{10} \frac{x}{318-x} = 0.127(t-1.46)$$

inserting the observed values of t and computing therefrom the cor-

² In computing the constants of the curve the data for the tenth, eleventh and twelfth months are not employed, since there is a possibility that in this region the curve of the first cycle may be distorted by growth due to the beginnings of the second cycle.

responding values of 0.127 ($t-1.46$) we can find, by referring to the above cited tables, the corresponding values of $\frac{x}{318}$ and, consequently, compute the "calculated" or theoretical values of x . The observed and calculated values are compared in table 3 and illustrated in figure 1, the smooth curve representing the calculated curve and the dots the weights actually observed. In this comparison the weights at ten, eleven and twelve months are included although the theoretical curve is not computed to fit these values. In the fifth and sixth columns of the table the divergencies between the actual and the theoretical weights are compared with the "probable errors" of the observed average weights computed in the usual manner from the variabilities and the numbers of infants weighed at each age.

TABLE 3
Males

AGE IN MONTHS	WEIGHT IN OUNCES		DIVERGENCE FROM OBSERVED AVERAGE	PROBABLE ERROR OF OBSERVED AVERAGE
	Observed	Calculated		
1	147.0	148.2	+1.2	±1.8
2	169.0	171.4	+2.4	±1.7
3	193.8	194.3	+0.5	±1.8
4	218.6	215.6	-3.0	±2.2
5	233.7	234.7	+1.0	±2.1
6	251.8	251.5	-0.3	±2.5
7	268.6	265.5	-3.1	±2.8
8	276.2	277.0	+0.8	±2.5
9	282.8	286.5	+3.7	±2.8
10	299.7	292.8	-6.9	±3.3
11	303.4	299.6	-3.8	±4.0
12	314.3	304.0	-10.3	±3.4

The corresponding equation for the first growth-cycle in females is:

$$\log_{10} \frac{x}{312-x} = 0.106 (t-1.54)$$

the comparison of the observed and calculated values of x follows in table 4 and is illustrated in figure 2.

It will be seen that the agreement between the observed and calculated values of x is excellent, the only important divergence being in the males at twelve months when, as explained above, deviations due to the intrusion of the second growth-cycle are to be anticipated.

TABLE 4

Females

AGE IN MONTHS	WEIGHT IN OUNCES		DIVERGENCE FROM OBSERVED AVERAGE	PROBABLE ERROR OF OBSERVED AVERAGE
	Observed	Calculated		
1	143.1	145.7	+2.6	±1.5
2	159.5	164.7	+5.2	±1.5
3	180.0	183.5	+3.5	±1.7
4	202.1	201.6	-0.5	±2.0
5	217.6	218.1	-0.5	±2.2
6	235.4	233.4	-2.0	±2.3
7	252.6	246.8	-5.8	±2.8
8	258.4	258.6	+0.2	±2.7
9	265.4	268.6	+3.2	±2.8
10	273.3	276.7	+3.4	±2.8
11	288.0	283.9	-4.1	±3.4
12	287.5	289.5	+2.0	±3.1

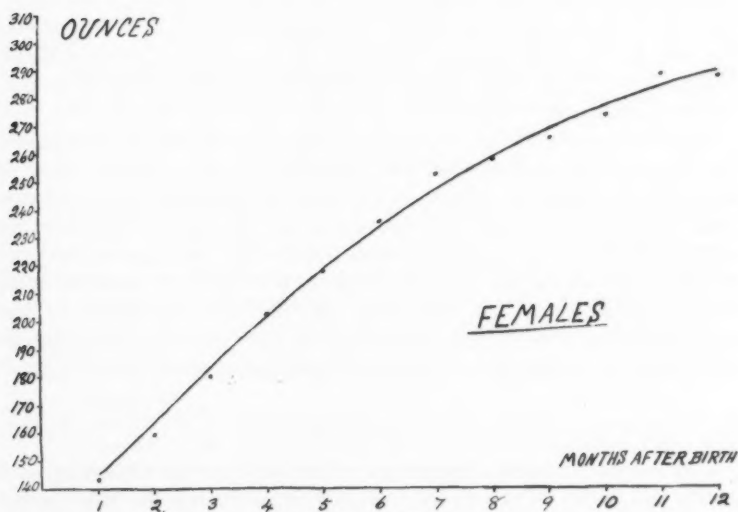


FIGURE 2.

In the following table (5) the values of the parameters A , $k \left(= \frac{K}{A} \right)$ and t_1 for British infants and for Australian infants of British descent (23) are compared:

TABLE 5

	MALES			FEMALES		
	A	$k \times 10^4$	t_1	A	$k \times 10^4$	t_1
British.....	318	399	1.46	312	340	1.54
Australian.....	341.5	398	1.66	350	317	2.47

It will be observed that while so fundamental a difference as that of sex has little effect upon the absolute magnitude ($= A$) of the first growth-cycle, environment has a pronounced effect, since one or two generations of the Australian (presumably more favorable) environment has resulted in an increase of from 7 to 12 per cent in the amplitude of the cycle. The specific velocity of the growth-process, however, ($= k$) is comparatively unaffected by environment, the identity of the two values for males being particularly striking, while it is decidedly less for females than for males. Corresponding with these correlations we find that the period of half-completion of the cycle (and maximum velocity of growth) is later in females than in males and in Australian than in British infants.

The form of the curve of growth in infants is therefore determined by two separate factors; the one, analogous to the absolute mass of reacting substances in a chemical reaction, is dependent upon environment and probably upon the abundance or deficiency of the habitual dietary; while the other, analogous to the specific velocity of a chemical reaction, is relatively if not absolutely independent of environmental or nutritional conditions and, being expressive of the nature of the growth-process itself as distinguished from the availability of materials for growth, is distinctively modified by sex and probably also by race.

4. SUMMARY

1. From data supplied by infant welfare associations in London and Leeds the curve of growth of British infants during the first twelve months succeeding birth has been constructed.

2. The Newman Standard, hitherto largely employed as a standard curve of growth of British infants, is defective in two particulars. In

the first place it makes no distinction between the sexes, although the growth-curves of the two sexes differ in very material particulars, and in the second place, especially during the early months, the weights indicated by the Newman Standard are considerably sub-normal.

3. The variability of male infants is somewhat greater than that of female infants. The variability of both sexes is greatest during the early months, or period of most rapid growth, and decreases towards the later part of the first year, when the rate of growth is also decreasing.

4. The curve of autocatalysis correctly represents the growth of British infants during the first nine or ten months succeeding birth.

5. Comparing the characteristics of the growth-curves of British infants with those of the growth-curves of South Australian infants of British descent, we find:

i. The absolute magnitude or amplitude of the first growth-cycle in man is unaffected by sex, but is decidedly affected by environment, being increased in the Australian environment by from 7 to 12 per cent.

ii. The specific velocity of the growth-process is, on the contrary, very little affected by environment and markedly influenced by sex, being, in both environments, decidedly lower in the females than in the males.

iii. The period of half-completion of the cycle is later in females than in males and in Australia than in England.

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STUDIES ON THE GROWTH OF MAN

IV. THE VARIABILITY OF THE WEIGHT AND STATURE OF SCHOOL CHILDREN AND ITS RELATIONSHIP TO THEIR PHYSICAL WELFARE

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In any fortuitously selected group of animals or human beings, the individuals comprising the group will be found to vary in greater or lesser degree from the type or average which the group collectively represents. When a large proportion of the individuals comprising the group differ from the average to a considerable degree we say that the group or type is highly variable, while when the converse holds good, that is when but few of the individuals comprising the group depart widely from the average, then we consider the group or type to be but slightly variable. It is evident that if a measure of variability can be conveniently obtained it represents a factor or group of factors which may conceivably be affected by environmental or physiological conditions in a distinctive manner. In comparative studies of growth under varying environmental or dietetic conditions a knowledge of the variability of the organisms studied is essential in order to be able to determine the deviations from the average which may be regarded as normal, and those which on the contrary must be regarded as abnormal and attributable to the experimental conditions under which we have placed the individuals which display them.

The measure of variability which is commonly employed by statisticians is that deviation from the average which one-third of the individuals display (1). This may conveniently be expressed as a *percentage* deviation from the average weight or stature or other measurement under consideration.

¹ I am indebted to Mr. R. M. Jewett for assistance in the computation and compilation of the data.

Investigations upon the growth of the white mouse (2) have shown that the variability in weight increases when the velocity of growth increases, decreases when the velocity of growth decreases, attains a maximum when the rate of growth attains a maximum, and attains a minimum, when the rate of growth sinks to a minimum, i.e., when the animal is fully grown. It has also been shown (3) that similar phenomena accompany the first year of post-natal growth in infants the variability of weight being relatively high during the early months when growth is relatively rapid, and relatively low during the later months when growth is relatively slow. Variability in weight is therefore affected in a distinctive manner by internal factors, namely the processes underlying and controlling the rate of growth. That variability may likewise be affected by dietetic factors is shown by the remarkable increase in the variability of mice when fed with unusual amounts of cholesterol (4); while, as I am about to show, the various factors comprised in the external environment may also exert an effect upon the variability of weight and stature in man.

As stated in the preceding article, it would appear probable that a high degree of variability is a condition which is disadvantageous to the collective welfare of the individuals which display it, since a high frequency of marked departures from the mean implies a more frequent overstepping of physiological limitations. Conversely, other conditions being equal, a disadvantageous environment might be expected to cause an increase in variability through enhancing preëxisting differences or at any rate preëxisting subnormality. It is, however, to be borne in mind that disadvantageous environmental conditions may cause a retardation of the growth of young animals which in its turn might be expected to lead to a *decrease* in variability. Where the disadvantageous conditions operate at a period of rapid growth, or of growth of which the rate is fluctuating, the retardation of growth due to unfavorable environmental conditions might be expected to lead to a diminution or subnormality of variability, while the same conditions, pressing upon an adult animal, or an animal growing at a relatively slow or steady rate, might be expected to lead to an enhancement or supernormality of variability.

From yet another point of view the investigation of variability is of importance. The growth or the physical well-being of an individual may be estimated in a variety of ways and by means of a variety of measurements of which weight and stature are those most frequently employed. If we desire to obtain a reliable criterion of abnormality

we shall do well to select for our purpose the measurement which is normally found to be least variable, since relatively small deviations from the normal are that case fraught with significance which does not attach to similar deviations from the normal displayed by a more variable characteristic. On the other hand if we desire to study in detail the effects of fluctuations in the diet, environment or physiological condition upon the dimensions of an individual, then we shall do well to investigate the more variable characteristics, since, from their high normal variability we may infer that they are exceptionally affected by environmental or other fluctuations. A previous knowledge of the relative normal variabilities of different physical dimensions in man or animals is therefore of service in enabling us to determine beforehand which type of measurement will be most likely to display the effects or correlations for which we are seeking.

The present investigation was undertaken with a view to ascertaining (a) the relative variability of weight and stature in school children and (b) the influence of environment upon the variabilities of these dimensions.

Through the courtesy of the director of health of the Oakland School Department, Dr. N. K. Foster, to whom I desire to express my very deep indebtedness, I have been permitted access to his very valuable and extensive records of the physical dimensions and welfare of the school children in the various schools in the city and environs of Oakland, California.

The records of fifty children of each sex and age, from 6 to 14 years inclusive (i.e., seventh to fifteenth year) were selected at random, excluding only seriously deformed individuals, from among the records of three "average" schools, that is, schools situated in localities inhabited by persons of average means and welfare. The heights and weights of these children were averaged and the variabilities of these dimensions determined in the usual manner (1).

The results are indicated in tables 1 and 2 and displayed graphically in figures 1 and 2.

From these results it is evident that the rate of growth in weight in both sexes increases continuously from the seventh to the fifteenth year and that the variability in weight undergoes a parallel increase during this period. There is a manifest tendency for maxima of variability in weight to coincide with maxima of yearly increment. The stature, on the other hand, increases during this period at an almost uniform rate and the variability of stature is correspondingly uniform.

TABLE 1

Males

AGE IN YEARS LAST BIRTHDAY	AVERAGE WEIGHT IN POUNDS	YEARLY INCREMENT	VARIABILITY	AVERAGE STATURE IN INCHES	YEARLY INCREMENT	VARIABILITY
			<i>per cent</i>			<i>per cent</i>
6	46.0		12.2	46.1		5.6
7	50.7	4.7	12.1	47.9	1.8	5.5
8	55.4	4.7	13.5	50.3	2.4	4.8
9	64.0	8.6	13.7	52.2	1.9	4.9
10	67.1	3.1	12.0	53.5	1.3	5.1
11	75.9	8.8	12.6	56.0	2.5	3.6
12	81.3	5.4	15.7	56.7	0.7	5.1
13	92.6	11.3	17.3	59.6	2.9	5.2
14	103.6	11.0	20.3	61.7	2.1	5.7

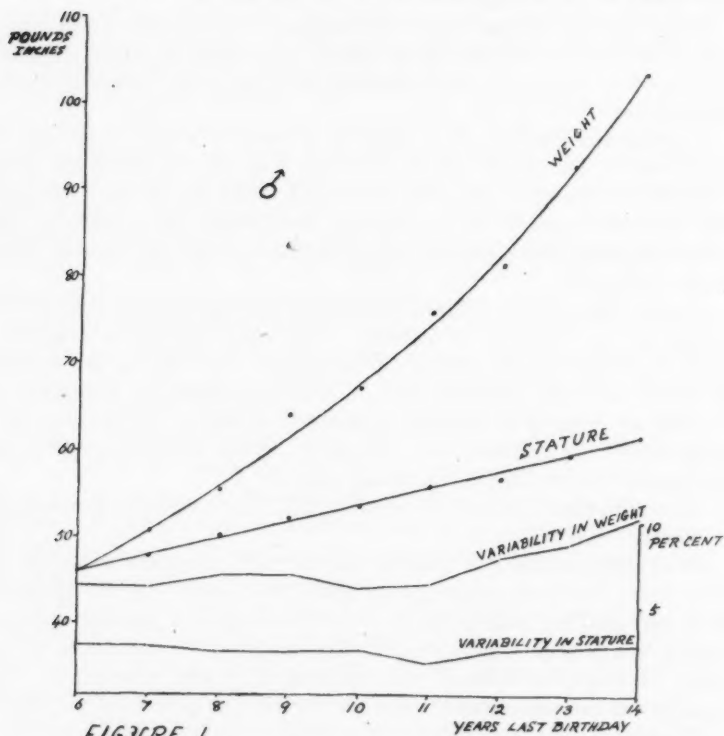


FIGURE 1

TABLE 2

Females

AGE IN YEARS LAST BIRTHDAY	AVERAGE WEIGHT IN POUNDS	YEARLY INCREMENT	VARIABILITY	AVERAGE STATURE IN INCHES	YEARLY INCREMENT	VARIABILITY
			<i>per cent</i>			<i>per cent</i>
6	44.4		11.9	45.3		5.8
7	49.4	5.0	12.0	47.7	2.4	4.4
8	53.5	4.1	13.4	49.8	2.1	4.1
9	59.7	6.2	12.8	51.4	1.6	4.6
10	66.1	6.4	14.2	54.2	2.8	4.8
11	75.2	9.1	20.3	55.5	1.3	5.8
12	80.7	5.5	17.1	58.0	2.5	5.2
13	97.3	16.6	20.5	61.1	3.1	4.7
14	105.6	8.3	13.6	62.4	1.3	5.4

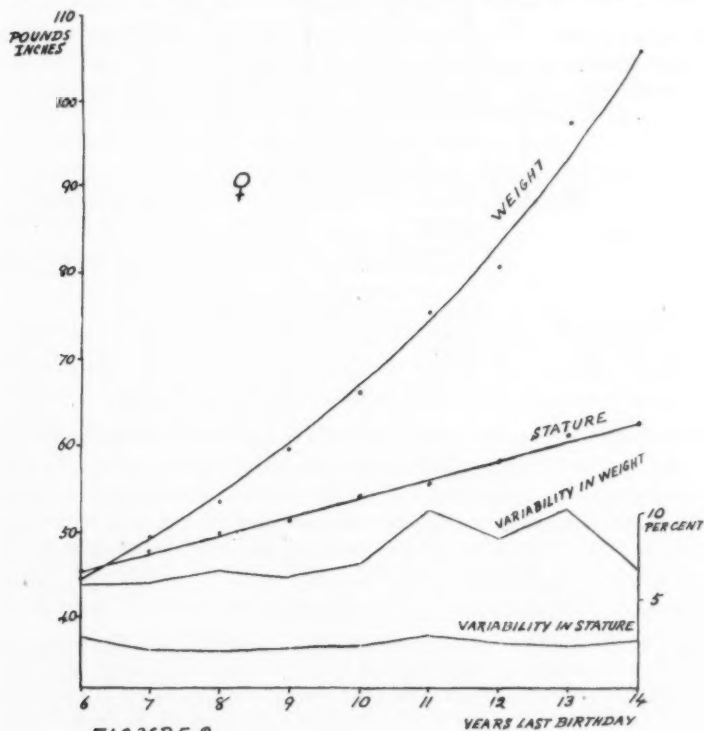


FIGURE 2.

The variability in stature is much less than the variability in weight, from which we may infer that as a criterion of abnormality the measure of stature is more reliable than that of weight, while as a sensitive indicator of the effects of environmental or dietetic fluctuations the measure of weight is to be preferred to that of stature.²

In order to directly estimate the influence of environment upon variability of weight and stature we also selected at random the records

TABLE 3

Males

SCHOOL	AVERAGE WEIGHT IN POUNDS	VARIA- BILITY	AVERAGE STATURE IN INCHES	VARIA- BILITY	PERCENTAGE OF CHILDREN HAVING	
					Infected adenoids	Adenoids removed
		<i>per cent</i>		<i>per cent</i>		
Residential district.....	59.6	14.2	50.8	4.2	10	34
Average district.....	55.4	13.5	50.3	4.8	16	6
Factory district.....	53.3	10.3	48.3	5.1	24	2

TABLE 4

Females

SCHOOL	AVERAGE WEIGHT IN POUNDS	VARIA- BILITY	AVERAGE STATURE IN INCHES	VARIA- BILITY	PERCENTAGE OF CHILDREN HAVING	
					Infected adenoids	Adenoids removed
		<i>per cent</i>		<i>per cent</i>		
Residential district.....	58.0	12.1	50.8	4.1	0	12
Average district.....	53.5	13.4	49.8	4.1	14	2
Factory district.....	51.7	11.7	48.5	4.5	10	2

of fifty children of each sex and of 8 years of age from among the records of children attending a school which was situated in a relatively poor industrial district, inhabited mainly by factory operatives, etc., while another group of records of fifty children of each sex and of 8 years of age was similarly selected from among the records of children attending a school situated in a relatively wealthy residential section of the city. The results are summarized in tables 3 and 4, while by

² As may be readily computed from the data tabulated above, the "standard deviation" or *absolute* as distinguished from *percentage* variability of stature shows tendency to attain a maximum at 14 years in the boys and at 11 to 12 years in girls. This coincides with results previously obtained by F. Boas, Report of the Commissioner of Education, Canada, 2 (1896-97), p. 1541.

way of indication of the degree of medical care received by the children of the three classes the percentage of cases of infected or operated adenoids is included in the tables.³

From these results it is evident that increasing unfavorability of environment results in a parallel increase of deficiency in weight and stature. It is a strikingly significant fact, also, that as the favorability of the environment decreases the proportion of medical care extended to the children, as indicated by the percentage of removed adenoids, also decreases, while the degree of medical neglect, as indicated by the percentage of infected and unoperated adenoids, undergoes a parallel increase.

The variability of weight, which is normally high, decreases in an unfavorable environment in consequence, as explained above, of the retardation of the rate of growth in weight which is also an effect of the unfavorable environment, while the variability of stature, which is normally much less variable, increases in consequence of the enhancement of normal deviations from the average which in this case more than compensates for the reduction of variability due to slackening of growth. We may infer that among children of this age a defect in stature and weight, with a high variability of stature and low variability of weight is indicative of the operation of an unfavorable environment.

SUMMARY

1. The rate of growth in weight of school children of both sexes increases from the seventh to the fifteenth year and variability in weight undergoes a parallel increase during this period. There is a decided tendency for maxima of variability to coincide with maxima of yearly increment in weight.

³ The parents of the children attending the school situated in the industrial district included a large proportion of individuals of foreign birth. It is not believed, however, that this factor invalidates the conclusions drawn from the above comparison, for on the one hand, there is no reason to suppose that the children attending the other schools were less heterogeneous racially than the children attending the school in the industrial district, for they represent in the majority of cases the offspring of persons whose parents were themselves of non-American nativity, and on the other hand, while racial heterogeneity in the absence of other factors might be expected to enhance the variability of every characteristic and dimension, the children from the industrial district were actually *less* variable in weight although more variable in stature than those from the average and residential districts.

2. The stature of school children during this period increases at an almost uniform rate and the variability of stature is correspondingly uniform.

3. The variability of stature is much less than the variability of weight, from which we may infer that as a criterion of abnormality the measure of stature is more reliable than that of weight, while as a sensitive indicator of the effects of environmental, physiological or dietetic fluctuations, provided statistical methods of investigation are employed, the measure of weight is to be preferred to that of stature.

4. Among children of 8 years of age it is found that increasing unfavorability of environment and lack of medical care results in a parallel increase in deficiency of weight and stature, accompanied by a decrease in the variability of weight and an increase in the variability of stature.

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- (4) ROBERTSON: *Journ. Biol. Chem.*, 1916, xxv, 639.

CONTRIBUTION TO THE PHYSIOLOGY OF THE STOMACH

XXXIX. THE HUNGER MECHANISM OF THE PIGEON AND ITS RELATION TO THE CENTRAL NERVOUS SYSTEM

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Some time ago Professor Carlson suggested that the restlessness of decerebrate animals might be correlated with the motor activities of the digestive tract. At the same time such preparations would serve as test objects for studying the relation of the gastric activities to the cerebral or other centers. Two lines of experiments have been carried out in studying these two questions.

METHODS.

Study of the changes in gastric activities was carried on by the method for graphic registration described by Professor Carlson (1) and by direct observation. The two methods have been employed as checks against each other in the effort to determine the advantages of each and the errors to which each is subject. Tracing No. 1 was made by holding the bird quietly in the hand and indicating the occurrence of contractions with a signal magnet writing against a moving drum.

The gastric apparatus of the pigeon is anatomically divided into three parts: crop, proventriculus or glandular stomach, and the muscular stomach or gizzard. The crop is described as a simple dilation of the esophagus and has a similar structure. [Details are given by Gadow in Bronn's Manual (2)]. The movements of the crop may be directly observed by plucking the feathers from the lower end of the neck and the upper part of the breast. To record these movements a small rubber balloon may be passed down the esophagus, but since this leads to persistent swallowing movements a better way is to make a fistula through which the balloon may be introduced, without inconvenience or discomfort to the bird. Since in pigeons it is unnecessary to take elaborate aseptic precautions for surgical experiments all that

need be done is to etherize the bird and make an incision through the skin and muscularis of the crop and insert a small piece of rubber tubing to keep the fistula open. If this tubing be removed the fistula will close in two or three days and the bird is none the worse for the operation. Two or three fistulae may be made in the same animal if needed. Direct observation of birds with and without such fistulae show that the presence of the small opening does not modify, or only very slightly, the normal movements of the crop.

Tracings of the movements of the proventriculus and gizzard in the normal bird may be obtained by pushing the balloon into the gizzard through a fistula made in the midline of the crop (fig. 2).

By the term decerebration in this paper is meant the removal of all the forebrain anterior to the thalamus. The technique of decerebration was developed by the trial-and-error method of experience. Practice showed that the principal difficulty lay in removing all of the forebrain without injury to the thalamus and cerebellum and without irremediably injuring the cerebral circulation. The layer of supraventricular cortex is very thin and that part of it which, in its position, corresponds to the occipital cortex of mammals is very easily overlooked until revealed at post mortem. Scradler (3) and Munk (4) state that about four-fifths of their completely decerebrated birds died shortly after the operation. In the present series of experiments, out of forty attempts only six birds satisfied the requirements of recovery from the "shock" of the operation, exhibition of typical decerebrate behavior, and post mortem confirmation of complete decerebration without trauma of the remaining parts of the brain. The technique finally adopted was as follows: In the etherized bird the cranium was exposed by a longitudinal incision of the skin. The bone overlying the cerebral hemispheres was then carefully removed, except that part directly over the median sinus. The dura should not be torn during this process. Then with fine pointed scissors the dura was laid open by the incision near and parallel with the medial and transverse sinuses. This was done for both hemispheres. All of this can be done without bleeding. A probe with a point curved to fit the posterior border of the brain was then inserted under the dura and the brain substance removed while the dura was left intact. Hemorrhage was controlled by gently packing with cotton. When the greater part of the bleeding had ceased, the cotton was removed, the cranial cavity left empty, and the skin was sutured over the bridge of bone that had been left in position. In successful preparations, after death

it will be seen that the edges of the dura have coalesced to form a sac filled with clear cerebrospinal fluid. All traces of hemorrhage have disappeared and the circulation of the remaining parts of the brain is as nearly normal as is possible after such an operation. Any post-operative bleeding is allowed to take care of itself. No dressing was applied in order to avoid pressure on the remaining parts of the brain.

LITERATURE

Descriptions and discussions of the behavior of decerebrate animals and bibliographies are given in all the better text- and handbooks. [See especially Schrader (3) and Tschermak in Nagel's Handbuch (5)]. Attention however must be called to the following points, significant for the present work.

Rolando in 1809 and Flourens (6) in 1822 first showed that decerebrated pigeons could be kept alive for long intervals of time.

Longet (7) showed that the behavior of such birds depended on whether or not the thalamus was included in the decerebration: that birds with the thalamus intact exhibited spontaneous movements, while those with the thalamus destroyed did not.

In striking contrast to the long continued discussions of the psychic or mental capacities of decerebrate animals, Vulpian (8) maintained that the "spontaneous movements are purely mechanical." "There is lacking in their reputed voluntary movements that capricious spontaneity that is seen in the normal animal." "Après l'ablation du cerveau au contraire ces mouvements sont dus a des excitations, soit exterieures . . . soit interieures et d'un certain ordre telles que la fatigue, la gene, que determine une meme attitude trop longtemps prolongee," etc. But he did not include visceral influences as a factor.

Schrader studied the influence of light, sound, and other external stimuli on these spontaneous movements and stated that hunger and thirst were factors. " . . . bei den automatischen Bewegungen die Erregungen den vegetativen Organen entstammen . . . die Bewegungen unseren Tauben von manchen vegetativen Reizen beeinflusst werden." But no objective proof of changes in the viscera was given.

Munk states specifically that the completely decerebrated bird with the thalamus intact moved about very little "so lange der Kropf nicht leer oder nahezu leer war. Aber war den Kropf leer geworden

so erfolgten jene ersteren Bewegungen (stretching, preening, etc.) häufiger und auch die Gehbewegungen wiederholten sich desto rascher, je länger die neue Fütterung unterblieb."

Goltz's (9) famous decerebrate dog was constantly restless during the day but quiet at night.

With reference to the activities of the gastric mechanism, Brown Sequard (10) noticed that rhythmic movements of the crop may be seen. Since then many physiologists have studied the intrinsic factors and the external nervous control of these organs. (Claude Bernard (11), Mangold (12), Stubel (13), Magnan, (14), Doyon (15).

Rossi (16) found that in the crop (*L'ingluvie*) two types of contractions occur: Periodic peristaltic contractions of the upper part and rapidly repeated rhythmic contractions of the lower part of the crop. These were recorded by the balloon method. He states that these contractions are not present when the crop is empty.

Doyon recorded similar contraction of crop by the balloon method. He noticed an alternation of the contractions occurring in the glandular stomach with those of the muscular stomach: that if the bird is hungry the crop is at rest, but distention of the balloon causes contractions. Also, that the crop is innervated by the vagus and electrical stimulation of the nerve causes a tonic contraction of the crop and rhythmic contractions of the gizzard.

Finally Kato (17) in a recent paper from Mangold's laboratory gives measurements showing that the pressure exerted by the contractions of the gizzard during hunger are greater than those occurring after feeding.

RESULTS

In this report the results stated for decerebrate birds are from birds which have been kept for one to four months after decerebration. For a few days after decerebration there may be a period of shock marked by vascular, temperature, and gastro-intestinal disturbances. These do not always appear, and, as pointed out by early workers, if they do not the "prognosis" is good.

Normal birds. When a relatively large balloon is put in the empty crop and then distended, the bird will frequently set up a shrugging sidewise movement that indicates that the balloon is a source of irritation. It shows very clearly that stimuli from the crop can induce changes in the behavior of the bird. This irritating action can always be produced by sufficient distension of the balloon. It is sometimes



FIG. 1. Decerebrate bird, second day of hunger. Beginning of contraction of crop indicated by down stroke of writing point; end of contraction by up stroke. Time in 10 second intervals.

seen after overdistension of the crop with food. These head and neck movements are of course not to be confused with contractions of the crop which may or may not be present at the same time. Avoiding these effects of overdistension there occur two types of contractions of the empty or nearly empty crop. First, strong peristaltic waves that run over either the entire crop, if it be empty, or only part of it, according to the degree of distension with food. Second, much more rapid rhythmical contractions at the extreme lower part of the crop. Both types are visible in the empty crop.

The frequency of the peristaltic waves and their point of origin varies with the volume of the contents of the crop. After feeding they seem to be entirely absent for a short time (30 to 45 minutes). Then at intervals there occur one or two contractions which can be detected by the balloon. In an hour or two there appear groups of three or four of these waves, and they gradually increase in frequency and vigor so that after five or six hours there may occur at intervals of a few minutes contractions in groups of six to twelve or more. By this time the bird is seeking food but if no food is supplied and the crop is allowed to empty, this same peristaltic or tonic activity continues. Sometimes the contractions are rapidly and continuously repeated for several hours, but they usually occur in groups periodically (fig. 3). Efforts to determine whether or not there was any change in the character of the contractions according to the duration of the starvation period showed that sometimes as the hunger period is prolonged (two or three days) continuously occurring contractions appear, at other times they do not. These variations occur in the same bird at different times.

To obtain tracings it was found necessary to keep the birds in darkened cages to keep them quiet. If while a period of contractions is occurring, a light be suddenly thrown on the bird the contractions cease immediately (fig. 4). A sudden noise will have the same effect. Attempts to capture and hold the bird in the hand at once inhibit the contractions. Spontaneous struggling of the bird of course does the same.

The contractions of the empty crop in the normal bird are not easy to demonstrate except by the balloon method. For the normal hungry pigeon in a cage is usually in a state of restless excitement, and it can be seen only that the crop is empty. But if such a bird be quieted in a partially darkened cage with the observers sitting quietly near, the contractions of the crop can be seen without any form of registering apparatus.

Probably one reason for the statement of other observers that the empty crop is quiescent is the failure to control these inhibitory influences. In a blinded pigeon but with the cerebrum intact it is much easier to observe these changes in the crop. The blind bird usually but not invariably sits quietly in its cage except when at intervals it is driven by hunger or thirst to hunt for food and water. In such a bird patience alone is required to discover that if the bird is quiet and the crop empty, at intervals one or more deep peristaltic waves will run over the entire crop and this is frequently the precursor of the bird's shifting its position or walking about the cage.

At other times instead of periodic contractions the entire crop may be so closely constricted as to nearly obliterate its lumen. It is evi-

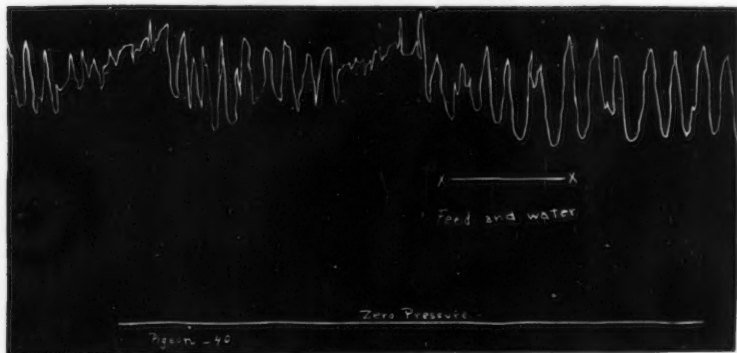


FIG. 2. Decerebrate bird, 24 hours hunger. Balloon in the gizzard.

dent that according to the explanation given by Cannon (18) of the relation between tonus and distension, peristaltic contractions will not appear on such a constricted organ. Between this condition of contraction of the whole organ and that of a partially relaxed crop over which run deep peristaltic waves, there may be found all intermediate gradations in the same bird at different times.

The rapidly repeated rhythmic contractions of the lower part of the crop occur at the rate of eight to ten per minute. Each of the much slower peristaltic waves of the empty crop requires ten to twenty seconds to complete its cycle. (Fig. 5.)

Very small amounts of water and food first stimulate and then inhibit the contractions. No inhibition by taste stimuli was found.

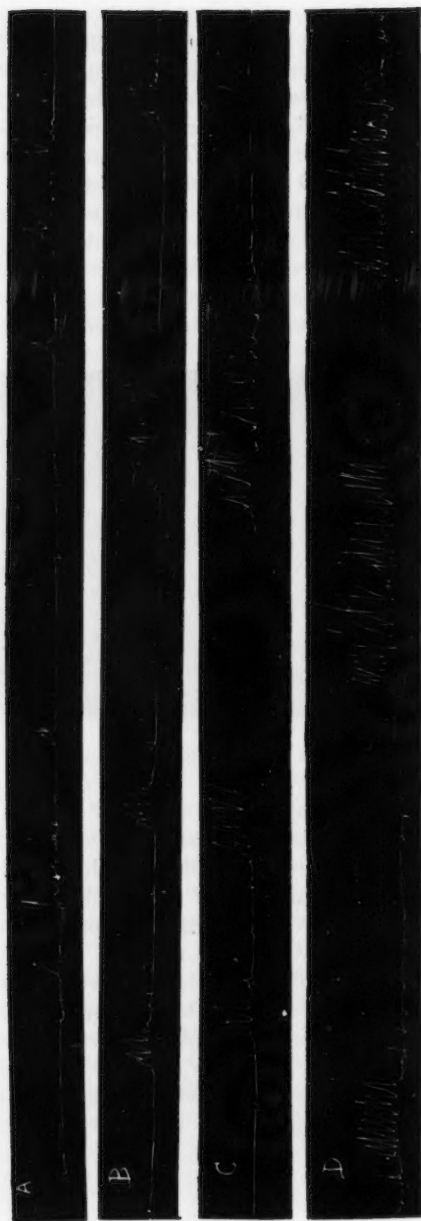


FIG. 3. Decerebrate bird. Contractions of crop. Parts of a continuous series. *a*, $\frac{1}{2}$ hour after feeding. *b*, 2 hours after feeding. *c*, 4 hours after feeding. *d*, crop empty 18 hours after feeding.

This is not surprising since these birds are dry-grain feeders. Lesions of the cerebellum or unilateral destruction of the semi-circular canals leads to a rather striking inhibition of the crop activities and hence stasis of food. This effect is more marked in birds with the cerebrum intact than in decerebrate birds, but it is true of both. The analogy to vertigo and seasickness in man with the accompanying gastric disturbances is obvious. Simple prolonged anesthesia or hemorrhage from the cranial arteries or sinuses does not have this effect.

Decerebrate birds. The preceding facts which with patience can be made out in the normal bird are more easily demonstrated in the decerebrate animal, for here inhibitory influences are at a minimum. They may be studied either by direct observation or by the balloon and graphic tracing method. As Doyon states, these contractions may be induced by distension with the balloon. But the balloon may be discarded and the still simpler device adopted of so pressing on one part of the crop with the fingers as to distend the remainder with the contained air. This recalls Auer's observation that in the nearly empty stomach of the rabbit distension with air induces lively contractions. But by either method, the response of the crop is periodic; it can not always be induced by simple distension but only at intervals. Evidently there is a periodic alteration of tone or of ability of the muscle to respond to the stimulus of distension.

These contractions occur either singly or in groups of two to twelve at intervals of ten to sixty minutes. At times they may run on continuously for an hour or more. Both types of grouping have been noticed in the same bird at different times. These contractions which have been spoken of as "spontaneous" by other workers might be designated the hunger contractions of the bird, for their appearance usually means subsequent restlessness of the bird.

One of the easiest ways of visibly demonstrating these contractions is to take a bird whose crop is empty and the bird restless and give it plenty of water. The crop relaxes, and the bird assumes the typical sleeping position. The crop slowly empties itself: its tonicity increases and when the bird again begins his wanderings deep contractions can be seen to be running over the incompletely constricted crop. Their occurrence can be understood as the resultant of that reaction between tone and distension which has been described by Cannon for the mammalian stomach.

If a very small amount of food or water is given this sets up a lively play of contractions in the crop. These soon disappear and there

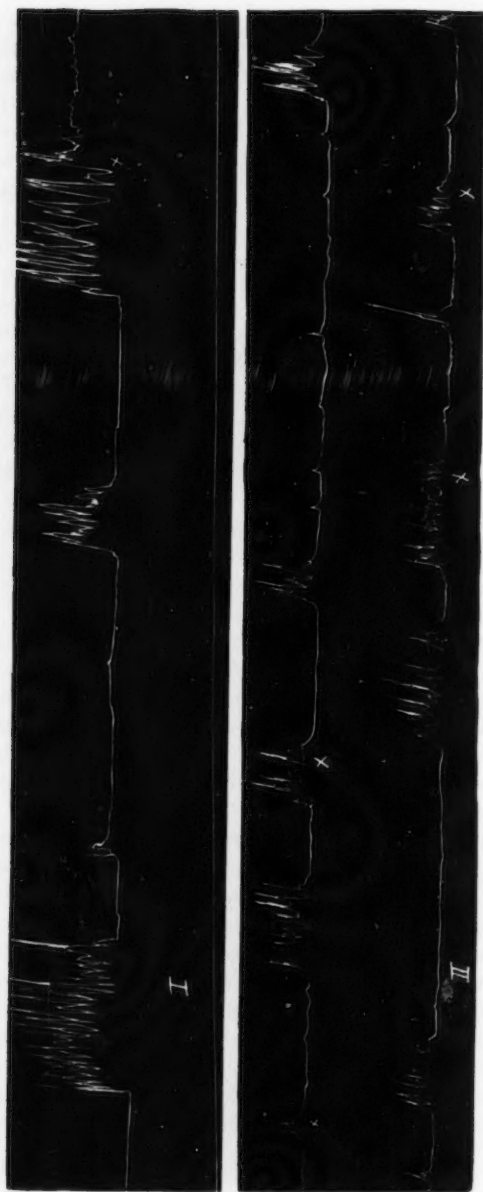


FIG. 4. Normal bird with fistula in crop. 24 hours starvation. I. At x bird was taken from cage by hand. II. Bird in a covered cage. At x cover lifted and light thrown on the bird. Time: length of each tracing is 20 minutes.

follows a marked relaxation of the previously constricted or contracting crop. This relaxation is considerably more than is necessary to barely permit the reception of the food swallowed. Solid food is a more efficient relaxing agent than an equal volume of water. With water there is some quantitative relation between the volume of fluid introduced and the relaxation of tone. Thus two to four cubic centimeters of water does not always have this inhibitory action, while eight to twelve cubic centimeters is followed by relaxation.

In the decerebrate bird which has only recently recovered from the shock of operation, light, noise, and handling the bird, have no inhibitory effect on the contractions. A month or two later, light and noise have no inhibitory effect, but handling the bird frequently stops the contractions. The reaction to food and water is identical with that of the normal bird. Lesions of the equilibratory apparatus have an inhibitory effect on the crop activities.

With reference to the motor activities of the proventriculus and gizzard tracings have been made which (in so far as the balloon method is justifiable) show that both of these parts of the hungry bird are vigorously and continuously contracting. As is shown by the tracing (fig. 2) periods of tetanic contractions occur.

With the thalamus intact, the conclusion seems unavoidable that when the hemispheres are gone, inhibitory reactions to external influences are gone but are still retained for intrinsic stimuli. So far as the gastric mechanism is concerned loss of the hemispheres means only that the gastric activities are no longer related to distant influences.

How do these factors relate to decerebrate restlessness? This restlessness is simply a continuous or periodic walking about. It is a familiar fact that in the dog after transection of the spinal cord, the stepping reflex may be easily elicited. The writer was somewhat surprised to find that in the dog after removal of the cerebral cortex with the thalamus left intact, any painful stimulus produced coördinated walking movements of all four legs (Sherrington, 19). Hence it seems fair to assume that in the bird decerebrate restlessness is only a reflex response to some stimulus. In such a bird, light and sound may be influencing factors but certainly are not the essential or adequate stimuli, for the blinded, decerebrate bird shows the same type of restless wandering as one with the eyes intact.

Moreover, whether or not in the decerebrate pigeon the restlessness is continuous or periodic with few exceptions depends on the size of the cage. A hungry bird which tends to wander around continuously



FIG. 5. Decerebrate bird. 24 hours since last feeding. In I, *a*, contractions of crop before feeding. *b*, just after feeding. In II, 24 hours hunger. Same as above except faster drum to show time interval of each contraction. Time in 5 second intervals.

in a large open space will on the same day react periodically in a smaller cage (20 by 30 inches). The hungry (not thirsty) bird exhibits periodic activity of the crop and periodic restlessness. The contractions of the crop are not caused by the restlessness of the bird for they run their course with the bird held quietly in the hand. And it has been repeatedly seen in the decerebrate birds and in blinded pigeons with brain intact, that the crop activity begins before the restlessness. Of course once started the restlessness tends to continue after the exciting stimulus has disappeared and may continue until fatigue sets in or until the bird is stopped by an obstruction. Cessation of the walking occurs only if the exciting stimulus is no longer present.

But the changes in the crop are not the only possible exciting factors, for it can be shown by feeding dry corn that the bird is subsequently restless and this can be ended by giving water. Furthermore, with plenty of food and water birds sometimes walk around for a short time, defecation follows and the bird becomes quiet. Evidently three possible stimuli inducing the walking reflex are the visceral impulses associated with thirst, hunger; and defecation. The last two are connected with hypermotility of the digestive tract. Conversely, by keeping the birds well fed and watered they seldom move about and in these occasional cases the movements can be understood as responses to fatigue or cutaneous stimuli.

If a normal pigeon is etherized and the viscera exposed the gizzard will be seen to be quiet, the intestine may or may not exhibit peristaltic contractions. If such a bird has previously received an intraperitoneal injection of one cubic centimeter of 0.1% pilocarpine sulphate the gizzard and intestine will be seen to be contracting vigorously. If the same dose be given to a decerebrate bird which has previously been fed and watered, in a few minutes the bird will either begin walking around or executing a rather comical stepping reflex without actually moving its position—a sort of “mark time” reflex, as it were. One-fourth cubic centimeter of pituitary solution (Armour) produces similar effects. Five-tenths to one cubic centimeter of 0.1% atropine abolishes the pilocarpine restlessness and likewise inhibits the contractions of the gizzard. The converse experiment may also be carried out. The decerebrate bird ~~is~~ given water but no food. While exhibiting the restlessness characteristic of hunger an injection of atropine will abolish the walking and the bird relapses into a somnolent position. This is suggestive but of course

not a conclusive line of experimentation. Drugs which stimulate the visceral activities cause restlessness in the bird. Drugs which quiet the viscera quiet the restlessness. Possibly stimulation of the central nervous system by the drugs might account for the changes in the response. But no stimulation for the central nervous system has been described for pituitrin and it produces visceral and reflex effects similar to pilocarpine. Atropine, if anything, is a stimulant of the central nervous system and yet it abolishes the walking response while at the same time quieting the viscera. Other drugs less powerful in action give varying results. Simple laxatives such as lactose and excess of water frequently cause restlessness with defecation but not always. These substances however do not cause increased tonus of the gut.



FIG. 6. Decerebrate bird, 24 hours after feeding. Balloon in lowest part of the crop. At *x*, water given by mouth. *b* is continuation of *a*. Time in 6 second intervals.

All of this suggests that two factors remain which if possible to control or estimate might reduce the behavior of the decerebrate bird to mechanical terms. These two factors are; threshold values for visceral stimuli and variations in the irritability of the central nervous system. It seems to the writer that if it is possible to reduce the study of visceral impulses to a quantitative basis the decerebrate animal is par excellence the test object for such work.

With reference to the reflex irritability of the central nervous system prolonged observation convinces one that variations do occur, although so far as the writer knows little has been done on the question. Further work is planned on this but the following may be noted here:

A hungry bird, not fed for several days, is restless during the day and quiet at night. This is not a question of illumination only, for the blinded decerebrate bird acts in the same way. However, this is not an invariable rule, for I have seen such birds periodically restless during the night and have seen these periods run parallel with periods of crop activities. But as a rule the bird is quiet at night in spite of changes in the digestive system. I have also seen that a louder noise is necessary to cause a reflex lowering of the feathers or opening of the eyelids when the bird has been fed than when it is hungry. The variations in irritability change so markedly as time is allowed for recovery from shock that a much more careful study of this will have to be made.

SUMMARY

In normal and decerebrate pigeons the crop when nearly empty and during subsequent starvation exhibits hypermotility or hyper-tonicity which is usually periodic but may at times run continuously for several hours.

In the normal bird this hyperactivity is inhibited by food, by water, by any conditions causing fear or surprise, or by labyrinthine and cerebellar lesions.

In the decerebrate bird (thalamus intact) water and food inhibit the hyperactivity. Inhibitory reactions to non-painful external stimuli are wanting but persist for intrinsic painful stimuli or lesions of the equilibratory apparatus.

Three kinds of visceral influences normally stimulate the restlessness of the decerebrate bird: Hunger associated with hypermotility of the gastric mechanism; thirst; and sometimes intestinal impulses. Pilocarpine and pituitrin augment visceral tone and contractions and induce the walking reflex; atropine quiets the viscera and abolishes the restlessness.

Variations in reflex irritability occur in the well kept decerebrate bird.

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SOME UNUSUAL EXPERIMENTAL LESIONS OF THE CEREBELLUM AND MEDULLA OBLONGATA

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In the course of some experiments on the semicircular canals and the otic labyrinth, a number of lesions of the cerebellum and the medulla oblongata resulted, either from slips in the experimental technique or from accident. They may be regarded as experiments contributing much to our own education while we were developing our operative methods. But as the results did not bear directly on the question of the effects of stimulation or extirpation of the labyrinth, they were not included in our first paper on the labyrinth (1). There is but a single lesion of each kind, except in the case of rupture of the posterior meningeal artery, but we present them at this time in the hope that some of the results may not be without interest to others who may have had similar accidents, and none of them are without a distinct interest to students of the physiology of the central nervous system. Some of the lesions were not such as one would care to duplicate more than necessary, and one or two, being the result of accident, might not be easily duplicated. The third one, on the lesion of the flocculus cerebelli, illustrates particularly well the statement, due, we believe, to Hitzig, that slight lesions may sometimes be more instructive than graver ones.

I. DEATH FROM HEMORRHAGE FOLLOWING INTRACRANIAL RUPTURE OF THE POSTERIOR MENINGEAL ARTERY

In the course of some experiments on section of the vestibular nerve intracranially, intracranial rupture of the posterior meningeal artery was followed by death in less than twenty-four hours. It had been observed in previous experiments that extracranial rupture of the artery had no noticeable effect. The method followed in sectioning

the vestibular nerve was to make a small trephine opening in the occipital bone over the medial edge of the petrous portion of the temporal bone and a little above the foramen of the posterior meningeal artery. As soon as the trephine had cut through the thinner part of the occipital bone at the medial edge of the temporal, the button of bone was removed and a probe inserted through the small opening in the occipital bone. The probe was passed downward and forward between the skull and the dura in the direction of the internal auditory meatus. In some experiments, the internal auditory meatus was not reached and the vestibular nerve not injured, as shown at post mortem examination. But irrespective of the success or failure of dividing the vestibular nerve, some of the animals were found dead the next morning. The immediate post-operative results were never more severe than in those experiments in which the labyrinth had been opened from the exterior, without disturbing the intracranial structures in any way. It would have been easy to attribute the deaths of our animals to shock, but our suspicions had already been aroused with reference to shock (2) as a cause of death and an error in procedure seemed much more plausible. Post mortem examination showed in each case, an extensive hemorrhage outside of the dura, never extending anterior to the tentorium, usually not extending beyond the median line of the skull, but extending downward and backward along the medulla oblongata. It is our opinion that death resulted from the pressure on the medulla due to the hemorrhage from the ruptured artery. Avoidance of the posterior meningeal artery or ligating it outside of the skull ended the trouble.

The death of the dogs seems to us to be of interest in connection with death from accidental fracture of the base of the skull in the human. Inquiry has failed to elicit any information on intracranial rupture of the posterior meningeal artery in these cases, several pathologists and surgeons saying that they never had seen a case. It would seem probable, however, that this artery is sometimes involved in the fracture, and its ligation, or the relief of pressure over the cerebellum and medulla by removal of the bone about the foramen magnum might afford some prospect of relief in a small percentage of cases of what is now a hopeless surgical condition. It is not to be expected that damage to the nervous tissue from internal hemorrhage will be very much decreased by operative procedures, but the increased intracranial pressure, which may in itself be a sufficient cause of death, would be relieved.

II. A FOREIGN BODY IN THE LEFT LATERAL LOBE OF THE CEREBELLUM

In one of these experiments, in exploring with a probe the operation wound in the bone resulting from removal of the labyrinth, the point of the probe slipped through into the cerebellar cavity and broke off. The condensed protocol of this experiment follows:

January 30, 1910. Black and tan dog. Removed left semi-circular canals. Point of dissecting probe broken off in bone and could not be removed.

After operation marked nystagmus in both eyes; appeared first in left eye.

February 6. Nystagmus disappeared on February 4. Today note:

1. Marked inclination of head to left side.
2. Cork-screw twist of head in cervical region.
3. If dog is lifted up, has marked tendency to fall to left.
4. When walking, dog inclines to left, and tends to go in small circles.
5. Drop reflex little affected.

6. Goes upstairs but refuses to come down; if induced to do so immediately falls over to left; and in one attempt rolled down the whole length of the stairs.

Dog killed.

Post-mortem. Piece of broken off probe was found imbedded in the left lobe of the cerebellum. No hemorrhage and no adhesion of dura mater to the cerebellum.

Fixed in Müller, with bone attached on left side.

The presence of the foreign body in the left lateral lobe of the cerebellum did not modify the type of nystagmus, nor appreciably prolong its course. Walking in a circle and falling over toward the side of the injury probably persisted slightly longer than they would after a purely labyrinthine lesion on one side.

III. LESION OF THE LEFT FLOCCULUS CEREBELLI WITHOUT INJURY TO THE VESTIBULAR NERVE

A slight lesion of the left flocculus cerebelli, without involvement of the eighth cranial nerve, did not cause nystagmus, torsion of the head nor rolling over to one side. The dog lay on the left side with left fore and hind legs extended and right legs often drawn up to the body. The head was outstretched and the nose on the floor. When placed on the right side, the dog attempted to stand up and fell over to the left.

The left eye was deviated outward and the left nictitating membrane was drawn to the median line. Oscillations of this eye occurred, but no constant movement could be made out. The right eye was not deviated and the right nictitating membrane was not more prominent than usual.

The dog became playful and was able to go up and down stairs, and to run about on icy walks, or to jump over his lead rope without any great difficulty, at the end of six weeks.

At the end of two months the dog was killed. A study of his reactions at this time showed that he still had a slight tendency to fall to the left. Often, when the dog did not fall completely over, he leaned to the left and the right hind foot was raised from the floor. The left forefoot swung inward in walking, particularly at the moment when the foot was raised from the floor at the beginning of a step. In starting to walk, he often went to the left before becoming able to go in a straight line. The drop reflex was manifested first in the left hind leg, but the right hind leg came down under him in a way better to break the fall. In walking, the left hind foot was out to the left, and the right hind foot nearly behind the left fore foot. There was some slight oscillation of the eyes on looking to the right, but it was inconstant. The eyes were more readily deviated to the right than to the left, but there was no noticeable nystagmus. The left eye was deviated a little to the left when the dog looked straight ahead.

The undoubtedly greater persistence of disturbances of locomotion and equilibrium, and the more enduring deviation of the eye, together with the fact that the homolateral eye alone was deviated, differentiate this lesion from a labyrinthine lesion. The fact that this dog lay preferably on the injured side is in direct contrast to the fact that a dog with a more extensive lesion, involving the lateral half of the cerebellum, lay preferably on the uninjured side.

The protocol of the experiment follows:

November 15, 1909. Young fox terrier dog. Operation on left side.

Trephine wound made lower down nearer to condyle and to lateral side of condyle on *left side*. Large vein opened and very considerable hemorrhage, stopped by removing bone and inserting cotton and exerting pressure.

The second trephine wound was made higher up. Through this the probe was inserted till the seventh nerve was found and then section was made. When the seventh was touched there was distinct contraction of the face muscles.

As the dog began to come out of ether there was noted (1) Twitching of the angle of the mouth on the left side, none on the right. (2) The nictitating membrane on the left side was drawn to the middle line; right side normal. (3) Corneal reflex marked in the right, very little in the left eye. No nystagmus observed.

November 15, 2 p.m. Dog found lying on left side in cage. Had moved some since he was first placed in the cage, but only a little. Made no sound when cage was approached and door opened. Wagged tail when petted and spoken to.

Nystagmus very doubtful; there was possibly a slow horizontal nystagmus

in right eye, but it is inconstant and more probably due to voluntary movements of the eye. Right eye is not deviated and right nictitating membrane is not more prominent than usual.

Left eye was deviated outwards and the nictitating membrane was drawn nearly to the median line of the eye. No constant movement could be made out in this eye.

The dog lay on his left side with left fore and hind legs extended, the fore leg more extended than the hind. Head outstretched with nose on the floor. Right fore foot usually drawn up and often right hind foot also. When placed on the right side, the dog tried to assume the erect position and then rolled over on the left side. Repeated. Occasionally left fore foot was stretched out as in decerebrate rigidity.

Right legs (fore and hind) were sometimes drawn up in inspiration, and tremors might occur toward the end of inspiration. Tremors became much more marked after the dog had lain uncovered for awhile, and might persist throughout expiration, though with diminished intensity.

The corner of the mouth on the left side did not gape.

November 21, 1909. Dog able to stand alone for a little time and to walk a short distance, but fell over to the left side. Left hind foot was away out to the left side in walking, and right hind foot was nearly behind left front foot.

November 28. Fell to left side. Hind limbs abducted; left eye deviated outward.

December 21. Dog demonstrated before joint meeting of the University of Chicago Biological Club and Chicago Neurological Society. Walked up and downstairs without falling, and ran over the slippery walks with ease.

January 16, 1910. Brought down for examination. In good flesh. Playful. Tendency to fall to left still present. Often, when dog did not fall completely over, he leaned to the left and the right hind foot was raised from the floor. The left fore foot swung inward in walking, particularly when the foot was lifted from the floor at the beginning of a step. In starting to walk, he often turned to the left before getting started in a straight line. When held up by the fore legs and "dropped" suddenly, the left hind leg was stretched out first and extended further out. The right hind foot came down under him better so as to be more efficient in breaking the fall.

Perhaps some slight nystagmus occurred when the eyes were directed to the right, but it was extremely slight and inconstant. One could not be certain of it. The eyes were more readily deviated to the left, but no nystagmus was observable. The left eye deviated slightly outward when the dog was looking straight ahead.

Etherized; 10 per cent formaldehyde in 0.9 per cent NaCl injected into carotid artery. Head removed, brain and upper half of spinal cord exposed and put into 10 per cent formaldehyde. There was a slight abrasion of the surface of the cerebellum along the path of the probe and some laceration of the flocculus.

IV. LESION OF THE MEDULLARY-PONTINE ANGLE ON THE LEFT SIDE

Unilateral rotatory nystagmus, loss of locomotor control, inability to stand, loss of the corneal reflex on the side of the lesion and a tendency to lie on the injured side were observed in a dog with a lesion at the medullary-pontine angle, involving the seventh, but sparing the ninth and twelfth, cranial nerves.

November 21, 1909. Fox terrier. Male. Rather large for fox-terrier.

Trephined at base of skull below occipital ridge and laterally to median line. Attempted to destroy eighth cranial nerve on *left* side. Severe hemorrhage. Cotton wool plug left in upper trephine hole. Wound sewed up and bandaged. No nystagmus observable at first, but nystagmus in the left eye was noticed about an hour after the operation was finished. The nystagmus in the left eye was rotatory, the longest diameter of the ellipse being in the vertical plane. There was no noticeable nystagmus in the right eye at this time. Given large hypodermic injection of morphine, as the animal seemed restless.

November 22, a.m. Dog lying on left side. Apparently had not struggled much, although a little cotton had been rubbed from the top of his head near the anterior edge of the bandage. Left eye deviated outward. Right eye looking straight ahead. Good corneal reflex in right eye, but apparently none in left. Did not try to have dog stand up as he seemed very weak.

November 23. Nystagmus present in left eye. Conjunctiva much injected. Eye washed out with boric acid solution.

November 25. Eye washed out again. Mouth showing symptoms of gangrene. Cornea covered with bluish white semi-opaque membrane. Lips on left side gape widely. No corneal reflex in left eye. Plug taken out of trephine hole near sinus under ether anaesthesia. Piece of iodoform gauze packing strip inserted in its place. Wound again bandaged.

November 28. Wound still open. Dog emaciated. Would lie only on left side. Paralysis of facial on left side. Unable to stand. Dog killed.

Post-mortem. Slight hemorrhage, extra dural, in cord. No hemorrhage in cerebral region. Hemorrhage in pontine and medullary region. Found large wound in medullary-pontine angle, involving facial nerve. Ninth to twelfth cranial nerves not affected.

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THE RESPONSES OF SINGLE MELANOPHORES TO ELECTRICAL STIMULATION

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PRELIMINARY

A considerable accumulation of evidence is at hand proving the melanophores of the lower vertebrates to be functionally modified smooth muscle cells. The responses of a variety of smooth muscle preparations to electrical stimuli exhibit certain characteristic peculiarities. If the melanophore is to be consigned to the category of smooth muscle, we should expect it to show the same peculiarities in its responses to electrical stimulation. In the absence of exact data concerning the reactions of single melanophores to electrical stimuli, and in the hope of confirming my previous conclusions, I have undertaken the experiments recorded in this paper. The results show that there is a close agreement between the responses of single melanophores and of typical smooth muscle preparations to faradic and galvanic stimulation. The evidence furnished by these experiments thus corroborates and amplifies the previous conclusion that the melanophore is to be considered a functionally modified type of smooth muscle cell.

PREVIOUS WORK UPON THE REACTIONS OF THE MELANOPHORES TO FARADIC STIMULATION

All the investigators (Buchholz (4), Pouchet (22), Lode (20), van Rynberk (29), Mayerhofer (21), von Frisch (30), Spaeth (26)) who have experimented with the effects of electrical stimulation in the skin of fishes agree that an induction current of sufficient duration and intensity produces a contraction of the melanophores. Such a contraction occurs whether the stimulus is applied directly to the surface of the fish or to an excised portion of the skin. Lode (l.c.) first ob-

served the contraction of the melanin granules under the microscope in the excised fin of medium-sized trout.

The responses of amphibian melanophores to faradic stimulation have been less constant, though much of the conflicting evidence is to be ascribed to incorrect experimental premises. The earliest investigators (von Wittich (31), Harless (12), and Hering u. Hoyer (13), found that a tetanizing current produces a lightening of the skin of *Rana esculenta* which was reversible, the skin subsequently resuming its original dark color. Bimmerman (2), Étérnod et Robert (8), Winkler (33), and Laurens (17) later made similar observations. Lister (19), Ehrmann (7) and others observed no effect from an induction current. The apparent discrepancy is not surprising since both Lister and Ehrmann considered the active phase of the melanophores to be the expansion. Their experiments were made upon light animals in which the melanophores were already contracted. With a strong tetanizing current the time required for the contraction of the melanophores in large larvae of *Amblystoma opacum* Laurens (l.c.) found to be at least fifteen minutes. Direct stimulation of small bits of excised skin gave similar results.

Faradic stimulation likewise produces a contraction in reptilian melanophores as was first shown by Bruecke (3) in the chameleon. The reaction in excised bits of skin is the same as in the intact animal. Both Bert (1) and Krukenberg (16) later verified Bruecke's observations. Keller (15), on the other hand, failed to observe any change in *Lacerta viridis* after stimulating with strong alternating currents. In the absence of any description of the experimental procedure it is impossible to judge the validity of this single negative instance.

From the foregoing briefly summarized experimental evidence, there seems to be no reasonable room for doubt that faradic stimulation produces a contraction of the melanophores of lower vertebrates. The exact nature of this contraction and its relation to other contraction phenomena has never been investigated.

EFFECTS OF FARADIC STIMULATION UPON SMOOTH MUSCLE

In the response to faradic stimulation both the latent period and the time required for a complete contraction may be said, in general, to be very much greater in smooth than in striated muscle. The effective liminal stimulus is also far greater for smooth muscle. This is

strikingly illustrated by the classical experiment of Fick,¹ who found that an induced current of sufficient strength to produce a powerful tetanus in a frog's gastrocnemius or in the hand of the experimenter could pass through an adductor preparation of *Anodonta*, in the same circuit, without producing any sign of a contraction.

Wide variations occur both in latent period and contraction time in different types of smooth muscle. The following table, modified from Gruetzner, shows the range of such variations in a few examples of vertebrate smooth muscle. The figures have only an approximate value, however, since in each type of smooth muscle considerable additional variation occurs with a change in the strength and duration of the stimulus.

TYPE OF MUSCLE	LATENT PERIOD IN SECONDS	DURATION OF A SINGLE RESPONSE. (TIME OF CONTRACTION AND RELAXATION IN SECONDS)	OBSERVER
Stomach muscle of frog....	1.5-10	60-120	Ranvier Schultz Gruetzner Winkler and others
Retractor penis of dog and horse.....	0.80-4.5	15-20 Contraction 90 Relaxation	Sertoli
Detrusor vesicae of cat....	0.25	5-6 Contraction 35 Relaxation	Stewart
Membrana nictitans of cat.....	0.5	5-15	Lewandowsky
Ureter of rabbit.....	Fraction of second to 1 second	2-7	Engelmann

EXPERIMENTAL PROCEDURE

In studying the responses of the melanophores of *Fundulus* to faradic stimulation the procedure was as follows. After the fish² had been washed several times in tap water, distilled water and 0.1 N NaCl,

¹ Quoted from Gruetzner (11).

² Throughout these experiments 8.5 to 10 cm. females of *Fundulus heteroclitus* were used.

or slightly modified Ringer solution,³ a few scales were removed to 0.1 N NaCl or the modified Ringer solution, where they were left until the melanophores were widely expanded. A single scale was then transferred to a shallow depression slide or a diffusion chamber and was there held in place by means of a bit of cover slip and a touch of vaseline. The stimulus was applied through non-polarizable electrodes of the Zn-ZnSO₄ type. Drawn out tufts of absorbent cotton or camel's hair brushes served as contact points which were brought to lie on either side of the scale, with their tips 3 to 6 mm. apart. The most satisfactory results were obtained when the scale was covered by a piece of supported cover slip only slightly larger than the scale itself. The supports prevented mechanical stimulation by contact with the cover slip and the small size of the latter eliminated the possibility of an asphyxiation error due to an inadequate supply of oxygen. In many of the earlier experiments the scale was held in place and stimulated by means of a small, movable, two-pronged fork of flattened platinum wires. The effects of the polar products of electrolysis were largely eliminated by a strong current of NaCl or modified Ringer solution which maintained the C_{OH} far below a stimulating minimum.

A series of preliminary trials showed that a record of the movements of the pigment granules in a single, unbranching process of a melanophore gave a fair picture of the contraction in the entire cell. All the graphs in the following pages represent the rate of movement of the most distal melanin granules in a single process of a single melanophore. Records of a series of responses in a single cell were always made by measuring the pigment migration in the same process.

RESPONSES OF THE MELANOPHORE TO FARADIC STIMULATION

Owing to the fact that the method of stimulation involves the passage of a considerable portion of the current through the mounting medium, it has proven impossible, up to the present time, to obtain a complete contraction of a melanophore as the result of a single make or break shock. It must, therefore, be borne in mind that the "single contractions" seen in the following curves are the result of a summation of make and break shocks.

A study of the responses of single melanophores to tetanizing currents of moderate intensity (fig. 1) brings out the following facts:

³ This modified Ringer was made up of 20 vols. 0.1 N NaCl + 1 vol. 0.1 N CaCl₂ + 1 vol. 0.1 N KCl.

1. The time required for a complete contraction and relaxation of the melanin granules in a process 0.1 mm. long is about of the order 90 to 120 seconds. This estimate is necessarily approximate since considerable variation occurs at the end of the relaxation phase.

2. The duration of the contraction phase is 25 to 30 seconds. Relaxation therefore requires from two to three times as long as contraction. The time relations for the contraction and relaxation in a single melanophore are thus approximately of the order seen in the case of a preparation of frog's stomach.



FIG. 1. Curve showing the response of a single melanophore to a brief tetanizing stimulus. The cell was removed to modified Ringer solution two hours and twenty minutes before stimulation began. The stimulus lasted nine seconds. After a latent period of 3 to 4 seconds the contraction began and lasted 21 seconds. The recovery was incomplete after 85 seconds. Upper line, time in seconds; middle line, stimulation period; lower line, time in minutes. $T =$ ca. 22°C . The steps in the rising phase of the curve are due to the method of recording the pigment migration.

3. The steps and other irregularities which frequently appear in the curves are due to the peculiarities of the pigment migration. This migration is rarely a perfectly smooth flowing of melanin granules. The pigment frequently becomes momentarily blocked or jammed in the cell process forming a minute, opaque mass, from the proximal side of which single melanin granules, or groups and lines of granules, may be seen progressing toward the center of the cell. Such blocks

usually last from one to several seconds, though in extreme cases careful focussing will reveal several thin lines of pigment granules moving towards the center of the cell from one or more outlying blocks after the contraction is apparently completed. This phenomenon is especially likely to occur when the melanophores have been removed from the fish for several hours.

THE ORIGIN OF THE CONTRACTION WAVE AND THE LATENT PERIOD

The study of a large number of contraction curves has shown that in the response to a tetanizing current of moderate strength at room temperature there appears a characteristic latent period of 4 to 6 seconds. Other observers have recorded somewhat longer latent periods in several species of fish. Thus Lode (l.c.) found in the case of the trout that the contraction followed $\frac{1}{2}$ to 1 minute after stimulating, whereas Mayerhofer (l.c.) noted a somewhat briefer latent period for *Esox*, 20 to 30 seconds, depending upon the strength of the current. In *Crenilabrus pavo* von Frisch (l.c.) found a distinct reaction in the melanophores 5 seconds after stimulating. It is significant that the latent period is in every case a long one, such as we might expect to find in a relatively sluggish tissue, like the smooth muscle of the frog's stomach.

By the latent period is meant the time that elapses between the application of the stimulus and the first sign of a contraction of pigment granules at the distal end of the process under observation. The above time relations apply only in the case of the distal pigment granules. The latent period for melanin granules lying near the proximal ends of the cell processes is less than half as great as for the distal granules. In other words the contraction wave begins at the center of the cell and spreads peripherally. The consistent difference in latent period at distal and proximal ends of the same processes in one melanophore is shown in the table below. The times were recorded on two stop watches, and different processes were selected in order to give the observations a random value.

Ex. Sp. 16-41. T. = 20°C. March 31, 1916.

9.00 a.m. Scales from a 10 cm. female to modified Ringer.

9.40 a.m. Stimulation began; 3 storage cells in series; secondary of Harvard inductorium at 6 cms., non-polarizable electrodes. Each stimulus lasted 6 to 10 seconds. Between stimuli the cell was permitted to reexpand fully. Contractions began after the following time intervals in seconds:

<i>Proximal</i>	<i>Distal</i>
2.0	5.0
1.8	4.4
1.8	4.4
2.0	6.0
2.2	4.4
2.0	5.2
1.6	6.2
2.2	7.8
1.6	4.6
2.2	5.6
Average.....2.0	Average.....5.4

The average distance between the proximal and distal points in the processes at which the migration first appeared was about 0.075 mm. The difference between the average times for the beginning of the contraction at the center and the periphery of the cell—i.e., $5.4 - 2.0 = 3.4$ —gives a value for the time required for the contraction wave to traverse a distance of 0.075 mm. A simple calculation shows that the speed of propagation of the contraction wave within the melanophore, under the above experimental conditions, is therefore only 22 μ per second, or 7.9 cm. per hour. This extremely slow rate seems to leave little room for doubt that we are here dealing with a passage of the stimulus through the substance of the melanophore and not along a broken branch of sympathetic nerve fiber. The origin of the contraction wave at the center of the cell suggests the possibility of the transmission of the tetanizing stimulus to the cell through a broken nerve fiber. The stimulus having reached the center of the cell, however, its further propagation appears to be independent of finer nerve fibrillae.

In the response to a tetanizing current of moderate strength, the peak of the contraction curve appears distinctly pointed, never rounded as in typical contraction curves of smooth and of striated muscle. An examination of the behavior of the melanin granules in a single process of the stimulated melanophore shows that near the end of the contraction phase a relaxation wave frequently originates at the center of the melanophore before the most distal of the pigment granules have completed their centripetal migration. At the proximal end of a process there thus appears a slowly travelling, distally moving wave of relaxation, which gradually encroaches upon and carries with it the proximally moving melanin granules. The sharp peak of the recorded contraction marks the point at which the relaxation wave has enveloped the last of the contracting pigment granules. In the



FIG. 2. Shortening of the latent period and increase in the height of the contraction following a decrease in the distance of the secondary coil from the primary. First curve 33 minutes after removal from the fish with secondary at 7 cm.; latent period, 8 to 9 seconds. Second curve 35 minutes after isolation from the fish, secondary at 5 cm.; latent period 3 to 4 seconds. Third curve 39 minutes after removal from the fish, secondary at 3 cm.; latent period 2 to 3 seconds. Time in seconds; $T = 24^{\circ}\text{C}$.

melanophore we thus find that the relaxation phase may be initiated before the completion of the contraction. Thus it appears that both contraction and relaxation originate at the center of the cell, though why this should be so is not at present evident. There may exist a correlation of some sort between this central origin of contraction and relaxation waves and the centripetal direction invariably taken upon stimulation by the pigment granules.

By comparing in a number of curves the time required for a com-

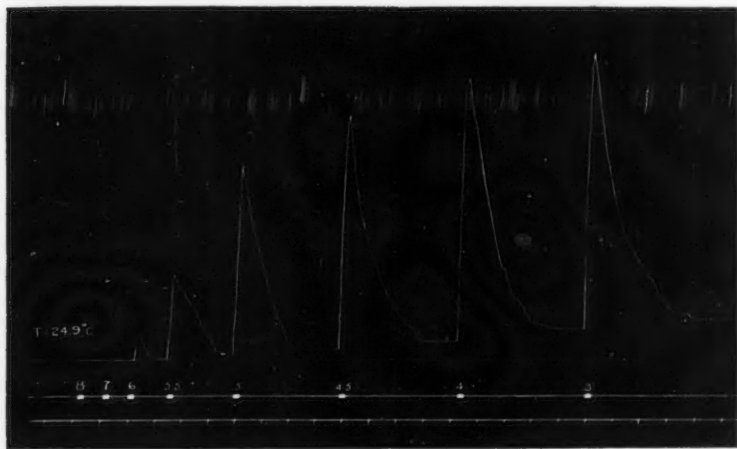


FIG. 3. Curve of increasing response with an increase in the strength of stimulus. The figures represent the distance in centimeters of secondary from primary. Stimulation began two hours and nine minutes after removing the cell from the fish. Time in minutes.

plete contraction and the actual distance traversed by the migrating pigment granules, it appears that the speed of the moving melanin granules is about 3μ per second or 1.08 cm. per hour. This is approximately one-eighth as fast as the propagation of the contraction wave (7.9 cm. per hour).

THE EFFECT OF INCREASING THE STRENGTH OF THE STIMULUS

Figures 2 and 3 show the effect of increasing the strength of the stimulating current by shortening the distance of the secondary from the primary coil. In figure 2 are shown three curves representing con-

secutive contractions in the same cell with the secondary at 7, 5 and 3 cm., respectively, from the primary. The stimulus lasted two seconds. The latent period becomes progressively shorter, falling from 8 to 9 seconds to 3 to 4 seconds and finally to 2 to 3 seconds. At the same time the speed and extent of the pigment contraction increases to the maximum shown in the third curve. Similar conditions obtain when a weaker stimulus is used.

Figure 3 shows the successive responses of the same cell to tetanizing currents of gradually increasing intensity. Owing to the slow rate of the drum the differences in the latent period are obscured, but the increased height of the contraction with the increase of the stimulus is very definite. These curves are strikingly similar to the series obtained by Stewart (p. 192, fig. 6, loc. cit.) upon increasing the stimulus in a preparation of cat's bladder.

SUMMATION OF CONTRACTIONS AND TETANUS

Whereas it has thus far proven impossible to obtain a complete contraction in a melanophore by a single make or break shock, by the proper spacing of a series of single break shocks⁴ which are individually almost or entirely ineffective, it is possible to obtain a typical tetanus curve.

Sertoli (25) found that a single induction shock was ineffective as a stimulus for the erector penis muscle. Stewart (l.c.) obtained a complete fusion of the responses to moderate break induction currents in the cat's bladder when the interval between the successive stimuli was reduced to one second.

The summation record for the melanophore (fig. 4) shows that with a shortening of the interval between stimuli, summation appears and becomes increasingly pronounced until, with an interval of 2 to 3 seconds, complete fusion occurs. Except for the time relations, this curve is precisely like the tetanus curve obtained from smooth and striated muscle preparations.

⁴ All the experiments in this series were carried out with the help of a modified Martin key. At the suggestion of Professor E. G. Martin the current was made and broken by means of a platinum needle dipping in a vial of clean mercury covered with alcohol. The contact spark was reduced to a minimum by inserting a small telephone condenser of 1 microfarad capacity across the primary terminals.

No refractory period has been observed in the melanophore, a stimulus being effective in every phase, both of the contraction and relaxation. Stewart (l.c.) has observed a similar absence of a refractory period in preparations of cat's bladder.

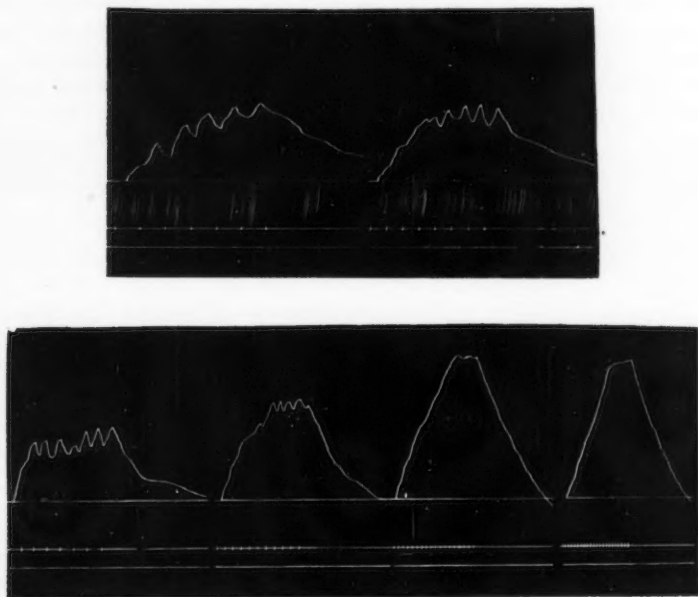


FIG. 4. Development of tetanus in a single melanophore. Powerful break induction shocks were applied at intervals of 15, 10, 8, 5, 3, and 2 seconds respectively. Stimulation began one hour and twenty-seven minutes after the removal of the cell from the fish. Time in seconds.

PREVIOUS WORK ON THE EFFECTS OF A CONSTANT CURRENT UPON THE MELANOPHORES

Experiments with the effects of a constant current upon melanophores are few in number and unsatisfactory in results. Lister (19) found that the melanophores of *Rana temporaria* failed to respond to galvanic stimulation. He believed that the absence of response in his experiments was only apparent and that, in reality, the chromatophores were paralyzed in the condition they happened to be in at the time of stimulation, thus eliminating the possibility either of a con-

traction or of an expansion. Hermann (14) could detect no color changes in frog larvae following galvanic stimulation. More recently Winkler (33) studied the effect of a galvanic current upon the melanophores of *Hyla*. A small piece of skin was transferred from the back of the animal to physiological salt solution and was then subjected to galvanic stimulation under the microscope. Under these circumstances Winkler observed an expansion of the melanophores—"schwarze Faeden" appearing from the deeper portions of the preparation. As to the reversibility of this process he states (p. 257):

Beim Aufhoeren des galvanischen Reizes verschwinden die Faeden; es ist aber dabei nicht zu entscheiden, ob dieses Verschwinden durch ein Zurueckziehen der Fortsaetze oder durch ein Fortwandern des pigments erfolge.

Presumably the finer details of a pigment migration were not visible in Winkler's preparation.

The relaxation of amphibian melanophores in isolated bits of skin observed both by Winkler (l.c.) and Laurens (17) following galvanic stimulation is probably to be interpreted as the effect of OH ions liberated at the cathode. Laurens (l.c.) has recorded an expansion both in whole larvae and in small, excised pieces of skin of *Amblystoma opacum* when stimulated by a constant current through platinum electrodes.

He says (p. 611):

When bits of isolated skin with contracted melanophores are thus stimulated on a glass slide with a current of from 3 to 4 m.a., and for about five minutes, the melanophores all expand. All attempts to demonstrate a greater effect of one pole in these expansion effects resulted negatively. . . . In two cases out of ten experiments with bits of isolated skin it was thought that the expansion at the cathode began earlier and was more extensive than at the anode, but this is not sufficient evidence for ascribing to that pole a greater effect.

From previous experiments with a variety of physiological stimuli (Spaeth, 26) I have been led to believe that the contracted phase of the melanophore is that of stimulation. It seemed strange, therefore, that a constant current should produce a *relaxation* in amphibian melanophores. Believing the polarizable electrodes of Laurens and Winkler⁵ to be a possible source of error in their experiments, I first made a number of preliminary trials with *Fundulus* melanophores using a

⁵ Presumably Winkler used polarizable electrodes since he makes no statement to the contrary.

constant current delivered through platinum electrodes. Scales immersed in 0.1 N KCl were subjected to galvanic stimulation from one, two and three 2-volt storage cells. The first experiment showed that, under these experimental conditions, a relaxation of the melanophores begins immediately. In subsequent experiments a drop of neutral red added to the KCl solution showed, furthermore, that the expansion invariably begins at the cathode. Under the low power of the microscope it was a simple matter to observe the color change of the neutral red at the cathode. The yellow area of alkalescence gradually diffused toward the anode and a short distance behind the periphery of the yellow semicircle the melanophores could be seen to be expanding. By the time the alkaline region had extended to the anode all the melanophores were either fully expanded or showed a distinct beginning of an expansion. When a scale was transferred from the now alkaline KCl solution to neutral KCl all the melanophores on the side of the scale towards the anode contracted completely. Thus in the region where the exposure to OH ions had been shortest and where the hydroxyl concentration was at a minimum the relaxation was reversible. All the melanophores in the region of the cathode, however, remained expanded and soon disintegrated (fig. 5). Fifteen minutes after having been returned to neutral KCl the scale appeared half dark and half light.

From this first set of preliminary experiments it seemed that when the melanophores are stimulated with a galvanic current, using platinum electrodes, the relaxation that appears is due, *not* to the electrical stimulus, but to the effect of OH ions liberated at the cathode. The following experiments supported this conclusion. Two depression slides were placed side by side, filled with $\frac{1}{2}$ cc. 0.1 N KCl and connected by a narrow bridge of filter paper, supported by a cover glass set on edge between the slides. A scale with contracted melanophores was now placed in each chamber. After adding a small drop of aqueous neutral red solution to the KCl solution in each chamber, a platinum electrode was brought into contact with the bony portion of

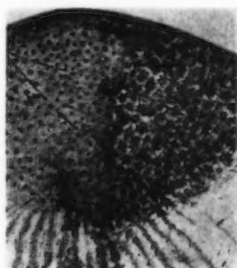


FIG. 5. Median portion of a scale showing the effect of stimulating with a constant current through platinum electrodes placed on either side of the scale. The melanophores towards the cathode are expanded by the action of the liberated OH ions.

each scale. A constant current (three storage cells) was now passed for five minutes from one scale over the moist filter paper bridge to the other. The diffusion from the cathode to the anode was largely prevented by the filter paper bridge. In about two minutes all the melanophores of the scale in the cathode chamber were fully relaxed, while those in the anode chamber remained contracted. The neutral red solution had meanwhile turned yellow at the cathode and was becoming colorless at the anode on account of the bleaching effect of the liberated Cl_2 or O_2 . At the close of the experiment, the scales and the electrodes were removed and, without disturbing the solutions in the two depression slides, a fresh scale with contracted melanophores was brought into each chamber. Fifteen minutes later, no current having passed



Fig. 6

Fig. 7

Fig. 6. Anodal products of electrolysis of KCl solution give no relaxation. See text for details.

Fig. 7. Hydroxyl ions produced at the cathode cause relaxation of melanophores in KCl. See text for details.

meanwhile between the chambers, all the melanophores of the scale in what had previously been the cathode chamber were completely expanded while those of the scale in the anode chamber remained contracted. The two scales were thereupon fixed in absolute alcohol. They are shown in figures 6 and 7.

From these two experiments it is evident that the expansion of melanophores which is initiated at the cathode upon stimulation with a galvanic current and polarizable, platinum electrodes is effected by the OH ions and not by the passage of the constant current through the cells. Finally, experiments were carried out with solutions of KOH in 0.1 N KCl which showed that, beyond a definite OH concentration, a relaxation of the melanophores invariably occurs.

THE EFFECT OF THE CONSTANT CURRENT ON SMOOTH MUSCLE

Practically all experimenters⁶ on smooth muscle preparations agree that both the make and the break of the constant current act as stimuli and produce contractions. The make contraction is larger than the break. Stewart (l.c.) has shown conclusively that in the cat's bladder the flow of the current also acts as a contracting stimulus. In his experiments the current (about 3 m.a.) was made, allowed to flow for a few seconds, and then broken. The result was a strong make contraction succeeded by a period of very slow, imperfect relaxation, the true relaxation occurring only after the break contraction. That the tonic elevation was actually due to the flow of the current was shown by making the current in the same way but shutting it off by means of a rheonome. In this way the influence both of flow and of break was eliminated. The curve showed a strong make contraction followed by a complete relaxation.

In spite of Stewart's proof to the contrary the impression continues to exist that smooth and striated muscle are alike in their response to the constant current. Thus du Bois Reymond (5) states (p. 554):

Bei der Reizung mit dem konstanten Strom gilt zunachst wie beim gestreiften Muskel, dass nur Schliessung und Oeffnung, jede als ein einzelner Reiz, wirksam sind, waehrend die Durchstroemung an sich keinen Reiz bildet.

In the case of the melanophores of *Fundulus* no response has as yet been observed to the breaking of the constant current, but both make and flow cause a contraction, as was the case in Stewart's experiments on the cat's bladder.

I have recently carried out a series of experiments upon the melanophores of *Fundulus* using non-polarizable electrodes of the Zn-ZnSO₄ type. Stimulation was effected by means of fine camel's hair brushes set in plaster caps covering the ends of the heavy glass tubes containing the concentrated solution of zinc sulphate. The plaster caps were made up fresh for every experiment or short series and in no case were the electrodes used longer than two hours at a time. The plaster was mixed with 0.1 N NaCl solution and, after setting, both electrode caps were saturated with 0.1 N NaCl. As an additional precaution the NaCl from the diffusion chamber, in which an experiment had been carried out, was tested for SO₄. The non-polarizable

⁶ Gruetzner (l. c.) gives an extended review of the older papers on this subject.

electrodes were most conveniently manipulated by having their adjustable uprights mounted on a sheet of glass (figure 3 of the previous paper in this number of this JOURNAL.) A depression slide or shallow diffusion slide containing about 0.5 cc. 0.1 N NaCl and a scale with expanded melanophores was brought below the objective on the sheet of glass on the stage of the microscope. The electrodes were then swung into position and the final adjustment of the single melanophore to be studied was accomplished by moving the sheet of glass carrying both the electrodes and the diffusion chamber. The direct current

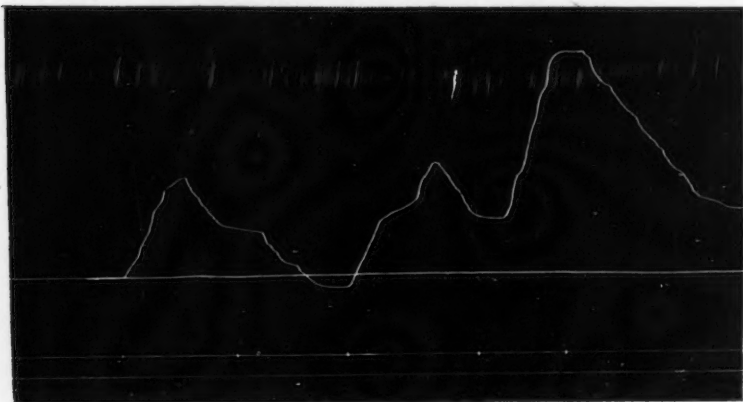


FIG. 8. Effect of a weak constant current through non-polarizable electrodes. Stimulation began 45 minutes after removing the cell from the fish to 0.1 N NaCl. Time in seconds. $T = 21.3^{\circ}\text{C}$. Detailed explanation in text.

of the city circuit was reduced by means of a long tube containing one movable and one fixed copper electrode and a dilute solution of copper sulphate. The strength of the current was varied by sliding the movable electrode back and forth in the tube. The actual current that stimulated the single cell could not be accurately measured since the electrodes simply dipped into the solution containing the melanophores.

The responses of the melanophores to a constant current of medium strength show a striking correspondence to the reactions of smooth muscle preparations under similar experimental conditions (fig. 8).

There is a sharp rise at the make after a very brief latent period

(<2 seconds) followed by a gradual and partial falling off of the contraction, giving a characteristic plateau. Stewart (fig. 9, p. 196, l.c.) recorded precisely this type of curve for the response of the cat's bladder. He noted further a distinct response in the bladder preparation when the constant current was broken, which I have never observed in the melanophore. There is, however, a latent period of about 10 seconds before the relaxation begins which may represent a suppressed influence of the break shock.

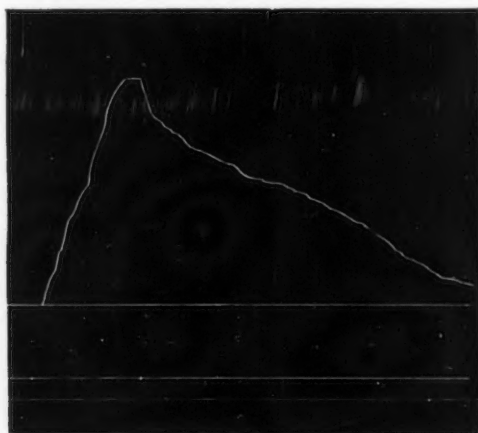


FIG. 9. Curve showing the response of a melanophore to a strong constant current through non-polarizable electrodes. A complete contraction occurs which continues during the *flow* of the current.

The second part of figure 8 represents the effect of the flow of the constant current. A stimulus of medium strength was sent into the preparation and, after the plateau had appeared (at the fourth signal), the current was gradually increased by means of the sliding electrode of the copper sulphate rheonome. In this case there was no additional make stimulus, yet the melanophore contracted completely and remained contracted during the flow of the stronger current. At the fifth signal the current was broken and a gradual expansion of the pigment followed.

When the stimulus is sufficiently strong the melanophore contracts completely and remains contracted until the breaking of the current.

This condition is illustrated in figure 9, which shows the effect of a strong constant current. The latent period is again very short (<2 seconds) and the contraction persists during the flow of the current, as is shown by the flat top of the curve. In this case the contraction represents the response to a combination of make and flow stimuli.

DISCUSSION OF RESULTS

The striking correspondence between the curves recorded here and the contraction curves of a variety of smooth muscles would not in itself be significant. The pseudopodia of an amoeba or a heliozoan might exhibit contraction phenomena of the same general order. However when this correspondence is considered in conjunction with other morphological and physiological characters of the melanophore such, for example, as the sympathetic innervation, the sensitivity to adrenalin and certain other drugs, the power of rhythmic pulsation, the ability to maintain a condition of increased tonus for protracted periods,⁷ etc., we are justified in attaching greater significance to the similarity.

In view of a considerable amount of verbal criticism regarding the earlier contention that the melanophore is to be considered a functionally modified smooth muscle cell it may be well to include here a brief statement of the author's position.

We apply the term "smooth muscle" to a variety of tissues which correspond to a number of empirically selected morphological and physiological characteristics. Investigation has shown that the melanophore conforms to *all* the criteria by which we recognize smooth muscle. Since the empirical criteria that establish the category of smooth muscle and the universal characteristics of smooth muscle are identical, it follows that the melanophore exhibits all the characteristics of smooth muscle. Hence it seems logical to speak of the melanophore as a type of smooth muscle cell. The insistence upon the nomenclature is significant in so far as further study of the melanophore may throw light upon certain obscure phases of the problem of muscle contraction.

SUMMARY OF RESULTS

1. The melanophores of *Fundulus heteroclitus* fail to contract completely when stimulated by a single make or break induction shock.

⁷ The melanophores in a living fish will apparently remain light over a light bottom indefinitely. I have kept *Fundulus* in a white aquarium for several months and have observed no tendency to turn dark during this period.

2. A tetanizing current of moderate intensity produces a complete contraction in which the rising phase lasts 25 to 30 seconds and the relaxation 65 to 90 seconds. Contraction and relaxation are completed in 90 to 120 seconds.

3. The form of the contraction curve is never smooth as in a curve of typical muscular contraction for the pigment granules exhibit a certain amount of irregularity and blocking in their migration within the cell processes.

4. The contraction wave originates at the center of the cell and passes to the periphery at the rate of about 22μ per second.

5. The most rapid pigment migration thus far observed in response to electrical stimulation is 3μ per second.

6. With a moderate tetanizing current there appears a latent period of about 5 seconds at the distal ends of the cell processes whereas nearer the proximal ends of the processes the latent period is only about 2 seconds.

7. With an increase in the strength of the stimulus there appears a progressive shortening of the latent period and an increase up to a maximum in the height of the contraction.

8. By the proper spacing of single break shocks which are individually almost or entirely ineffective as contracting stimuli, there appears a summation of stimuli. Eventually, with an interval of 2 to 3 seconds, complete fusion occurs, giving a typical tetanus curve.

9. The relaxation of the melanophores observed by previous investigators upon stimulation with a constant current and polarizable electrodes is probably brought about by the hydroxyl ions liberated at the cathode and is not the result of galvanic stimulation.

10. By using non-polarizable electrodes it appears that both the make and the flow of the constant current act as contracting stimuli. No response to the breaking of the constant current has thus far been observed.

11. In its responses to electrical stimulation the melanophore shows all the characteristics of smooth muscle. The experiments recorded in this paper thus corroborate and amplify the author's former conclusion that the melanophore is to be considered a functionally modified type of smooth muscle cell.

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A DEVICE FOR RECORDING THE PHYSIOLOGICAL RESPONSES OF SINGLE MELANOPHORES

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In a previous paper¹ I have figured a curve showing the rhythmic pulsations of a single melanophore of *Fundulus* following a ten minute immersion in a 0.1 N solution of BaCl₂. This curve was obtained by measuring, with an ocular micrometer scale, the greatest diameter of a melanophore in the course of an hour, at intervals of from ten to thirty seconds. These empirical diameters were plotted against the time, giving a curve of pulsation. By using several microscopes with similar micrometer eye-pieces it was a simple matter to compare the effects of different stimulating solutions upon melanophores of the same fish. When the experiments lasted several hours and the movements of the pigment granules were relatively sluggish, this method proved more convenient than the automatic method described below. Frequently, however—as for example, in the case of a contraction by KCl or an induction current—the responses were so rapid that it became impossible to obtain an exact record of the pigment migration in terms of a series of diameters.

In order to obtain more accurate records of the physiological activities of the melanophores, the following graphic method was devised. The movements of the pigment granules in a single process of a melanophore were followed with an ocular micrometer carrying an adjustable scale. A cell was selected in which the processes were relatively long and straight. A single process was then brought to lie at right angles to the division of the micrometer scale with an empirically selected scale division just tangent to the outermost pigment granules (fig. 1a). When distal and proximal movements of the pigment granules occurred they were easily followed by turning the adjustment screw of the ocular micrometer. By means of a thin belt the turns of the adjusting screw

¹ Spaeth: Journ. Exper. Zool., 1916, xx, 193.

were transmitted to a light set of pulleys and thence, by means of a cord winding about the pulley axis, to a heart lever and kymograph. A record of the pigment migration within a single process of the melanophore was thus obtained.

In every experiment a scale bearing the superficial layer of melanophores was immersed in the experimental solution contained in a diffusion chamber. The first type of chamber was made of glass slides. A 50 by 75 mm. slide formed the bottom; the sides were made of 25

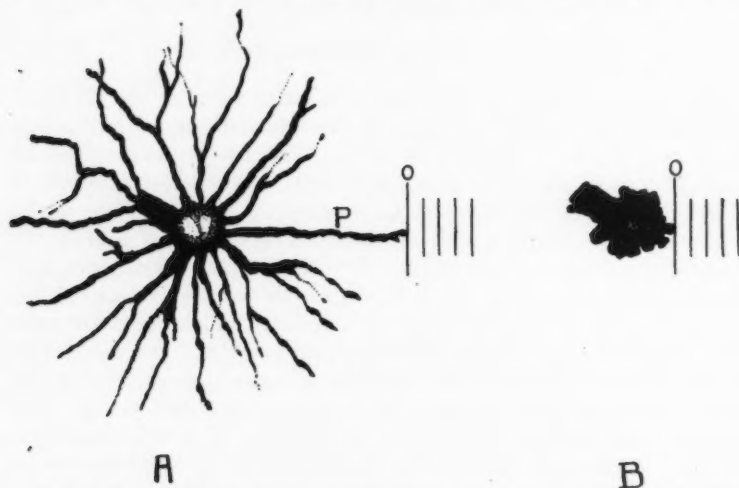


FIG. 1. Diagrammatic representation of the appearance of a cell just before stimulation, (A) and after the contraction is complete, (B). The division of the eye-piece micrometer marked *O* is kept tangent to the most distal pigment-granules of the process *P* throughout the contraction by turning the adjusting screw of the ocular micrometer.

by 75 mm. slides and the ends of cut slides of the same width, bound together by a modified Dehktinsky cement which was found to have no appreciable effect upon the melanophores. A bent glass tube (not shown in the figure) to which a small rubber hose and clamp were attached, served as an outlet, while fresh fluid could be added either (1) directly, by a pipette, in cases where a measured quantity was desired, or (2) by means of a tube and funnel attached at a level of about 10 cm. above the bottom of the chamber. The position of a scale carrying the melanophores to be observed could be changed

by means of a mechanical stage which carried the entire chamber. All observations were made with a 7 Leitz objective used as a water immersion lens.

In order to maintain a constant base line it was necessary to devise a mechanism for holding the melanophores immovably in position throughout the period of observation. This was accomplished as follows. A piece of fiber-composition was cut to span one end of the diffusion chamber, leaving a short flange projecting over each side. By means of a horizontal set screw (fig. 2 b) in one of these flanges, the bridge was firmly clamped across one end of the chamber. In the middle of the bridge a vertical set screw (fig. 2 c) raised or lowered the free end of a thin strip of spring brass (fig. 2 d) attached to the

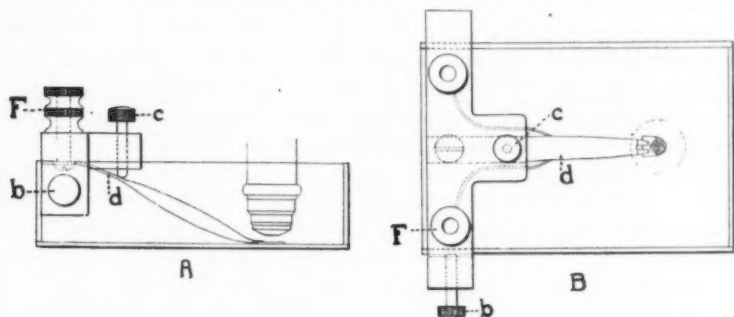


FIG. 2. Diffusion chamber and method of holding a scale in place during an observation. See text for details.

lower surface of the bridge. The free end of the brass strip carried a small two-pronged fork made of heavy platinum wires. Figure 2B shows how a scale is held in place against the bottom of the diffusion chamber by means of the flattened tips of the platinum wires. The platinum wires serve not only as a clamp for holding the scale with the melanophores in position but also as electrodes for faradic stimulation. Short pieces of copper wire were fused in a blast lamp to the proximal ends of the platinum wires and attached to two binding posts (F) on the fiber bridge. The two platinum wires were mutually insulated by means of a short glass rod lying between them. This rod was attached to them by cement and was similarly fastened to the free end of the spring brass. In order to avoid, as far as possible, the short circuiting of the secondary current through the salt solution between

the prongs of the fork, the flattened ends of the platinum wires, with the exception of the lower surface of the tips, were coated with a thin layer of cement.

A second type of diffusion chamber was found to be very convenient in experiments demanding a rapid change of solutions. This consisted of a circular glass chamber with glass inlet and outlet tubes mounted on a heavy slide. The scale was kept in position in the middle of the circular chamber by means of a small piece of cover glass about 3 by 8 mm., one end of which rested upon the free, bony portion of the scale. By a touch of vaseline² the other end was made to adhere firmly to the bottom of the chamber. This arrangement was found particularly useful when stimulating with non-polarizable electrodes.

The movement of the ocular micrometer scale is normally accomplished by turning a milled head. This head was replaced by a small brass crank which enabled the operator to obtain a continuous and smooth motion of the micrometer scale. The rotation of the ocular micrometer screw shaft was transmitted to a set of wooden pulleys by a light belt. A piece of three-strand fish line with the ends spliced together gave very satisfactory results. The pulleys were so arranged that, in addition to the three transmission speeds, there were two winding spools for the cord carrying the heart lever. Various combinations of transmission speeds and winding pulleys gave magnifications on the kymograph drum ranging from 940 to 3850 diameters.

The device for carrying the writing lever was constructed as follows. A lead block (fig. 3, *L*) about 4.5 cm. long, carrying a heart lever, was arranged to run on parallel brass rods (*R*) the ends of which were screwed into a small iron base. A heavy piece of sheet brass (*S*) was soldered across the upper ends of the brass rods. By maintaining a constant distance between the rods, any possibility of sticking or of lost motion in the lead block was prevented. Through a hole in the middle of the brass piece (*S*) a cord (*C*) was passed and attached to an eye in the lead block. The other end of this cord was fastened to the winding pulley by a touch of sealing wax. By turning the adjusting screw of the ocular micrometer, the lead block carrying the writing lever was raised and lowered by the winding and unwinding of the cord attached to the pulley axis. The advantage of this arrangement as compared to an ordinary heart lever lies in the fact that since the writing tip moves in a plane perpendicular to the base line, every

² The vaseline has no injurious effect on the melanophores.

point of the contraction curve may be projected to the time-interval line, and, since it is unnecessary to apply corrections for a lever arm, the progress of the experiment may be read directly.

In experiments with salt solutions it was found that better results were obtained by momentarily raising the objective from the surface of the scale when the solution was being changed. The raising of the objective would obviously release the tension on the transmission

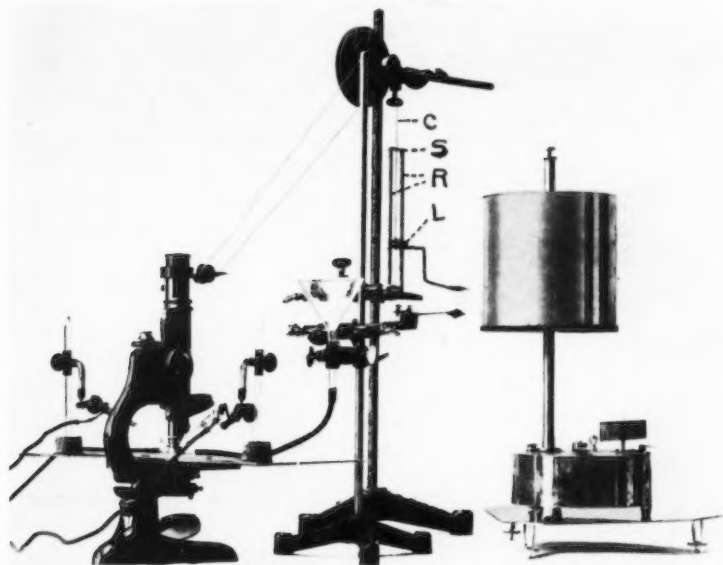


FIG. 3. Recording apparatus arranged for stimulation with non-polarizable electrodes. Letters explained in text.

belt, and the weight of the lead block would move the writing lever out of position. In order to maintain the position of the writing point, even when the objective was raised above the surface of the solution, a spring brake was attached to the pulleys, adjusted to overcome the rotary tension of the lead weight at the pulley axis.

At the beginning of an experiment the time and stimulation signals were aligned with the moveable writing lever as follows. The tip of the writing lever having been brought into contact with the lower

edge of the drum, the eye-piece micrometer screw was turned until the writing lever had traced a single vertical line upon the smoked paper. The time and stimulus signals were now brought into contact with the kymograph paper with their tips on this vertical line. The angle of the vertical line with the base line was adjusted to 90° by means of a levelling table carrying the kymograph.

The most obvious disadvantage of this apparatus is that it requires constant attendance and manipulation by the operator, which, in addition to being time-consuming and laborious, introduces a personal equation into every observation. In most cases, however, the reactions are relatively so slow that, with a little experience, the pigment may readily be followed with the micrometer scale division. The advantage of the apparatus lies in that it affords an opportunity for recording graphically the physiological responses of single melanophores. Such graphic records and a discussion of their significance in the problem of muscle contraction will be found in another paper in this number of this journal.





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